Design, Synthesis, and Biological Activity of Novel, Potent, and Selective (Benzoylaminomethyl)thiophene Sulfonamide Inhibitors of c-Jun-N-Terminal Kinase

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Received November 27, 2003

Several lines of evidence support the hypothesis that c-*Jun N*-terminal *k*inases (JNKs) play a critical role in a wide range of disease states including cell death (apoptosis)-related and inflammatory disorders (epilepsy, brain, heart and renal ischemia, neurodegenerative diseases, multiple sclerosis, rheumatoid arthritis, and inflammatory bowel syndrome). The screening of a compound collection led to the identification of a 2-(benzoylaminomethyl)thiophene sulfonamide (AS004509, compound I) as a potent and selective JNK inhibitor. Chemistry and structure−activity relationship (SAR) studies performed around this novel kinase-inhibiting motif indicated that the left and central parts of the molecule were instrumental to maintaining potency at the enzyme. Accordingly, we investigated the JNK-inhibiting properties of a number of variants of the right-hand moiety of the molecule, which led to the identification of 2-(benzoylaminomethyl)thiophene sulfonamide benzotriazole (AS600292, compound **50a**), the first potent and selective JNK inhibitor of this class which demonstrates a protective action against neuronal cell death induced by growth factor and serum deprivation.

Introduction

The c-*Jun N*-terminal *k*inases (JNKs) (also known as 'stress-activated protein kinases') are members of the mitogen-activated protein kinase (MAPK) family together with p38 mitogen-activated protein kinases (p38 kinases) and extracellular signal-regulated kinases (ERKs). MAP kinases are serine/threonine kinases that are activated by dual phosphorylation of the threonine and tyrosine residues of the Thr-X-Tyr segment located on a loop adjacent to the active site.[1,2] The activation of each MAP kinase is carried out by a specific MAP kinase kinase. Activated MAP kinases phosphorylate various substrates, including transcription factors such as c-Jun, ATF-2, Elk1, NFAT, p53, and a cell death domain protein,[5−8] which in turn mediate the response to stimuli by regulating the expression of specific sets of genes. Accordingly, members of the JNK family of kinases are activated by the proinflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), as well as by environmental stress, such as that induced by anisomycin, UV irradiation, hypoxia, and osmotic shock.[3]

Three distinct genes encoding JNKs have been identified (*jnk1*, *jnk2*, and *jnk3*), and at least 10 different splicing isoforms exist in mammalian cells.[4] JNK1 and JNK2 are widely expressed in a variety of tissues, while in contrast, JNK3 is selectively expressed in the brain, heart, and testis.[4,9] Each JNK isoform binds to its substrates with a different affinity, suggesting that substrate specificity is the central regulatory element of JNK-dependent signaling pathways in vivo.[4]

In terms of the physiological function of JNKs, mice lacking *jnk1* or *jnk2* exhibit deficits in T-helper (CD4+) cell function.[10−12] Double knockout animals are embryonic lethal, although fibroblasts from such animals are viable in vitro and exhibit a remarkable resistance to radiation-induced apoptosis.[13] The *jnk3* knockout mouse exhibits resistance to kainic acid-induced apoptosis in the hippocampus and to subsequent seizures.[14] It therefore appears that JNK activity is critical for both the immune response and for programmed cell death,[15] and that therapeutic inhibition of JNKs may provide clinical benefits in a wide range of apoptosis-related and inflammatory disorders such as neurodegenerative diseases, reperfusion injury, multiple sclerosis, and rheumatoid arthritis.[16] Along the same lines, recent evidence further suggests that JNK inhibitors may also prove beneficial in the treatment of vascular, metabolic,[17] and oncological diseases.[18]

An increasing number of JNK inhibitors have entered development pipelines in the past few years,[16] such as the anthrapyrazolone SP600125, which is an ATP-competitive JNK inhibitor that exhibits moderate selectivity in a range of Ser/Thr- and Tyr- protein kinases assays.[17,19] Another important contribution to the identification of JNK inhibitors has been made very recently by Merck researchers, who have published the JNK3

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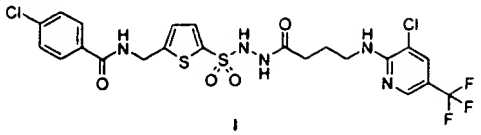
10.1021/jm031112e CCC: $27.50
Published on Web 11/26/2004

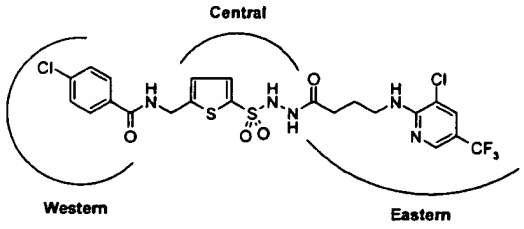
Table 1. Kinase Selectivity Profile of Initial Hit Compound I



I

kinase	JNK3	JNK2	JNK1	p38	MEK1	ERK1	PKC	PI3K	AKT	GSK3	P56Lck	EGF
IC_{50} (μM)	0.3	1.1	1.36	>10	>10	>10	>10	>10	>10	>10	>10	>10

Chart 1. SAR Mapping of Screening Hit I (AS604509)



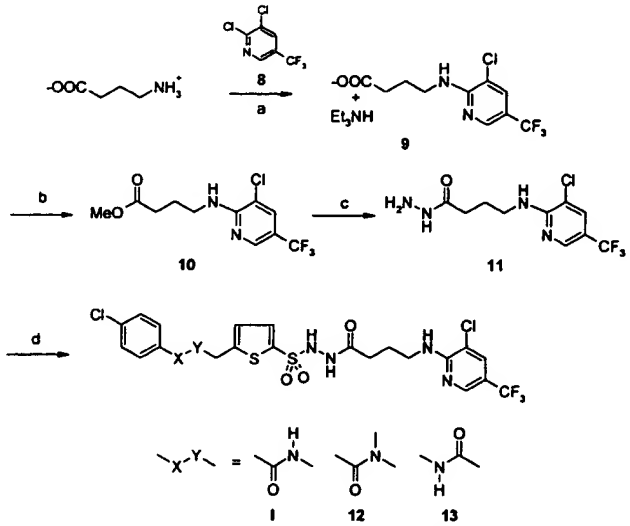
crystal structure complex with various JNK small molecule inhibitors.[20]

In view of the above, we initiated a drug discovery program aimed at identifying and characterizing novel, small molecule JNK inhibitors. Here we report on a class of (benzoylaminomethyl)thiophene sulfonamide JNK inhibitors[21] that demonstrate high selectivity for the enzymes in question when tested in a panel of serine/threonine and tyrosines kinases (Table 1). The starting point for this work was the identification of compound I in high throughput screening, which led us to initiate SAR investigations around the "western", "central", and "eastern" parts of the molecule (Chart 1).

Chemistry

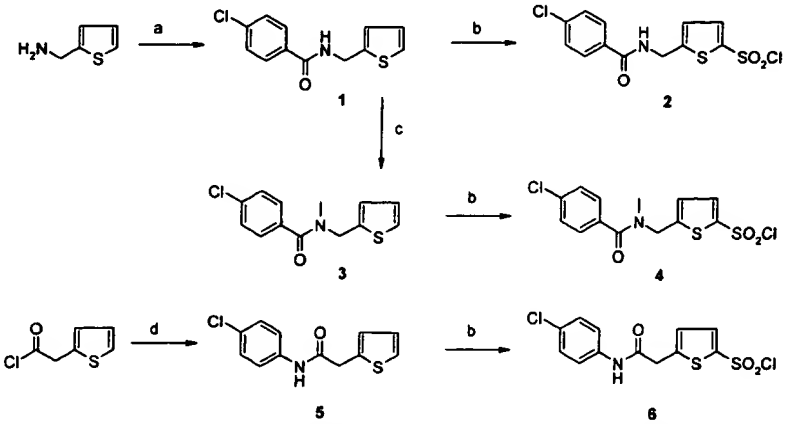
The synthesis of compound I was carried out on a multigram scale, without chromatographic purification of the intermediates (Scheme 1). Commercial 2-aminomethylthiophene was benzoylated and treated with chlorosulfonic acid to provide the sulfonyl chloride **2** in moderate yield.[22] This reaction could be applied to the synthesis of the closely related sulfonyl chlorides **4** and **6**. On the other hand, γ-aminobutyric acid was treated with the 2-chloropyridine (**8**) at 100 °C in an autoclave to provide carboxylate **9**. Esterification and treatment with hydrazine provided the acyl hydrazide **11** in 59% yield. This was coupled to the sulfonyl chlorides **2**, **4**, and **6** under optimized conditions (pyridine, $CHCl_3$) to yield the acyl hydrazides **I**, **12**, and **13** in crystalline form (Scheme 2). Initial SAR studies were focused on point-modifications of inhibitor **I**. The *N*-sulfonyl-*N'*-acylhydrazine moiety in **I** was replaced by a glycineamide, considered as a bioisostere, by reacting sulfonyl chloride **2** with glycine-*tert*-butyl ester in quantitative yield, followed by an ester-deprotection using 25% TFA in dichloromethane. Intermediate **14** was then coupled to the appropriate ethylenediamine **15** (PyBOP/DIEA), affording **16** in almost quantitative yield.

Scheme 2[a]



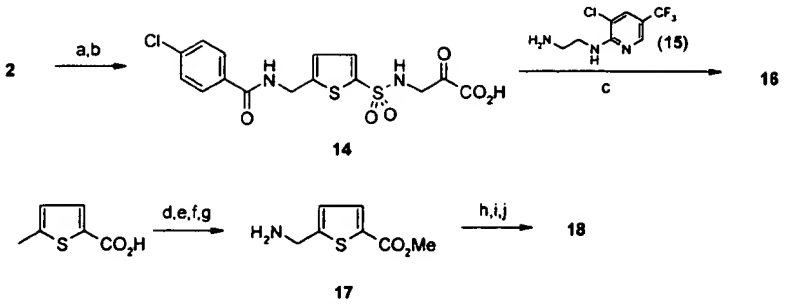
[a] Reagents: (a) Et_3N, MeOH, 100 °C; (b) H_2SO_4, MeOH, reflux (59%); (c) hydrazine, MeOH, reflux (76%); (d) **2**, **4**, or **6**, Py, $CHCl_3$, reflux (40–94%).

Scheme 1[a]



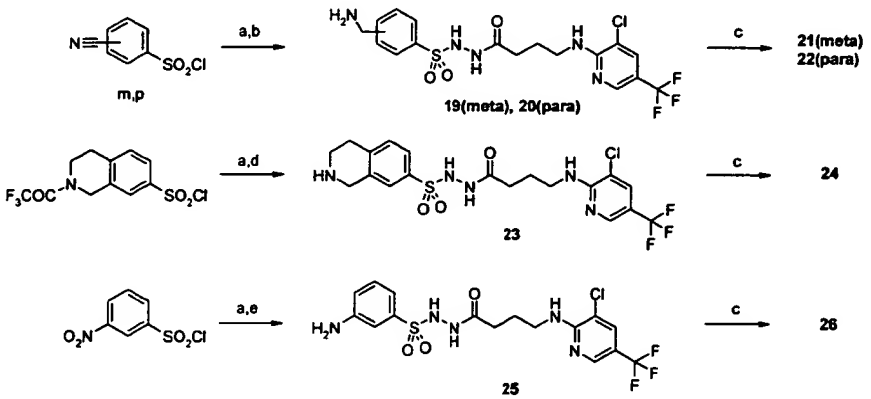
[a] Reagents: (a) 4-chlorobenzoyl chloride, DIEA, DCM, 0 °C (98%); (b) HSO_3Cl, rt (43–63%); (c) NaH, DMF, 0 °C (98%); (d) 4-chloroaniline, Et_3N, DCM (86%).

Scheme 3[a]



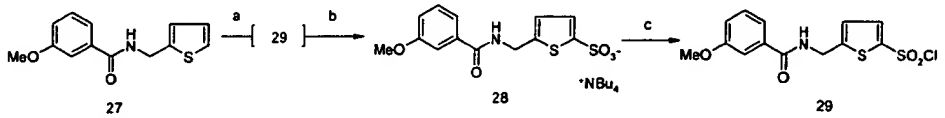
[a] Reagents: (a) Glycine-*tert*-butyl ester, DIEA, DCM, 1h (95%); (b) 25% TFA in DCM, 1 h (quant.); (c) **15**, PyBOP, DIEA, DCM, 15h, rt (95%); (d) H_2SO_4/MeOH (2 M), reflux, 2 h (89%); (e) NBS, dibenzoylperoxide, CCl_4, reflux, 24 h (98%); (f) NaN_3, DMF, 80 °C, 2 h; (g) H_2 (50 psi), Pd/C, EtOH, 15 h (50%); (h) 4-chlorobenzoyl chloride, DIEA, DCM, 3 h (90%); (i) LiOH·H_2O, THF/water, 5 h, 60 °C (98%); (j) **11**, DCI, HOBt, DCM, 3 h (60%).

Scheme 4[a]



[a] Reagents: (a) **11**, pyridine, $CHCl_3$, reflux, 2 h (97%); (b) $LiAlH_4$, THF, 30 min (67–87%); (c) 4-chlorobenzoyl chloride, DCM/Py 30:1, 3 h (35–78%); (d) K_2CO_3, MeOH, 50 °C, 1 h (97%); (e) $SnCl_2$·$2H_2O$, DMF, 15 h (36%).

Scheme 5[a]



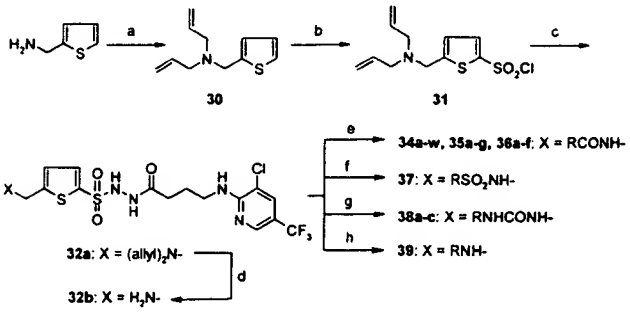
[a] Reagents: (a) HSO_3Cl, THF; (b) NaOH, Bu_4NOH (97%, two steps); (c) $(COCl_2)_3$, DMF, DCM (60%).

The carbonyl analogue **18** was synthesized starting from commercially available 5-methylthiophene-2-carboxylic acid. Intermediate **17** could be accessed over four steps without purification in 34% yield. The methyl group on 2-methylthiophene was converted into an aminomethyl group by a bromination/azide nucleophilic replacement/hydrogenation sequence[23] to give intermediate **17**. Ester **17** was then saponified and coupled (DCI, HOBt) to hydrazide **11**, affording **18** in moderate yield (Scheme 3).

In a second stage, modifications on the central thiophene ring were addressed. Replacement of the thiophene moiety by a furan heterocycle was performed using chemistry analogous to the one described in Schemes 1–3 and is not further discussed here. Bioisosteric replacement by a benzene ring, substituted in either the meta or para position, started from the corresponding commercially available cyanobenzenesulfonyl chlorides. Meta- and para-substituted cyanobenzenes were coupled to hydrazide **11** under reflux, followed by nitrile reduction and ultimately acylation, leading to compounds **21** and **22** with good overall yields. First efforts to rigidify the flexible aminomethyl moiety were undertaken by shortening the linkage to an anilino derivative and later by replacing the core structure by a tetrahydro-isoquinoline moiety. The aniline derivative **26** was accessed by coupling 3-nitrobenzenesulfonyl chloride to hydrazide **11**, followed by reduction ($SnCl_2$-dihydrate), and by acylating the aniline intermediate **25** (overall yield 75%). The tetrahydroisoquinoline analogue **24** was synthesized starting from its commercially available trifluoroacetyl derivative, which was coupled quantitatively to hydrazide **11**, followed by deprotection using potassium carbonate in methanol. The free amine intermediate **23** was acylated as above (Scheme 4).

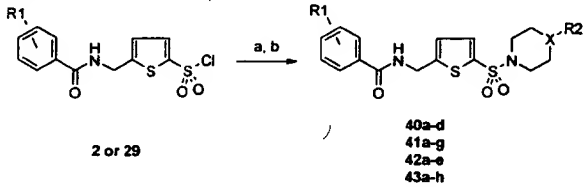
The route to sulfonyl chlorides **2**, **4**, and **6** could not always be successfully applied to analogues carrying substituents others than *p*-chloro. For instance, treatment of *m*-methoxybenzamide **27** (Scheme 5) with HSO_3Cl gave a gummy reaction mixture, and partial HSO_3Cl-catalyzed hydrolysis during workup lowered the yield. Accordingly, it was preferable to deliberately induce hydrolysis (1 equiv of aq NaOH) and to extract the sulfonic acid as its tetrabutylammonium salt (Bu_4NOH/DCM).[24] The nicely tractable sulfonate **28** was

Scheme 6[a]



[a] Reagents: (a) allyl bromide, DIEA (80%); (b) *t*-BuLi, THF, −78 °C; SO_2, −78 °C to rt; NCS (53%); (c) **11**, Py, $CHCl_3$, reflux (98%); (d) *N*-dimethylbarbituric acid, $Pd(PPh_3)_4$, DCM (67%); (e) RCOCl, pyridine:THF 1:5, −40 °C (50−80%); (f) RSO_2Cl, Py:THF 1:5, rt (50%); (g) RNCO, Py:THF 1:5, rt (70%); (h) RCHO, $NaBH(OAc)_3$, DCE, rt (97%).

Scheme 7[a]

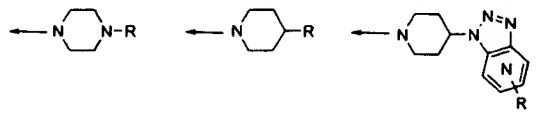


[a] Reagents: (a) polymer-bound morpholine (4 equiv), amine (1 equiv), DCM/DMF, 15 h, rt; (b) polymer-bound aminomethylbenzene (2 equiv), polymer-bound isocyanate (2 equiv), 5 h, rt (60−98%).

readily converted to the sulfonyl chloride **29** with triphosgene in the presence of catalytic amounts of DMF.[25]

The route to hit compound **I** was modified again for the exploration of the SAR around the 4-chlorobenzamido motif. 2-Aminomethylthiophene was protected as its bisallyl derivative **30** (allyl-Br, Et_3N) and converted into the thien-2-ylsulfonyl chloride **31** via metalation (i. *t*-BuLi, −78C. ii. SO_2. iii. NCS).[26] It is important to note that while chlorosulfonylation of **27** under 'standard' conditions with HSO_3Cl provided 2% of the unwanted thien-3-yl regioisomer, treatment of **30** with *t*-BuLi/SO_2/NCS afforded in contrast less than 0.5% of the thien-3-yl isomer. **31** was then coupled to the corresponding acyl hydrazide **11** to yield the bis-allyl aduct **32a**, which after deprotection ($Pd(PPh_3)_4$, *N,N'*-dimethylbarbituric acid)[27] gave the primary amine **32b**.

Chart 2. Focused Set of Western End Replacements

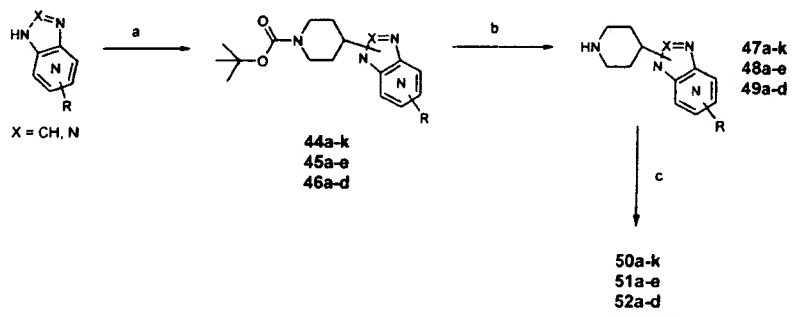


The latter was treated with a slight deficit of an acyl chloride (Scheme 6, route e), sulfonyl chloride (route f), or isocyanate (route g), respectively, to provide the amides **I**, **34a−w**, **35a−g**, **36a−f**, the sulfonamides (e.g. **37**), or the ureas **38a−c** in typically 82−100% optical purity (254 nm). **32b** could also be reductively alkylated using the corresponding aldehydes in the presence of $NaBH(OAc)_3$ (route h) to afford secondary amines (e.g. **39**).

The *N*-acyl-*N'*-sulfonylhydrazine moiety was perceived undesirable as far as 'druglike properties' are concerned (high MW, low permeability, N−N-linkage, high number of rotatable bonds).[28] Efforts were therefore directed at replacing this portion of the molecule. The sulfonyl chlorides **2** and **29** were reacted in parallel with a number of amines. These syntheses were carried out in solution using polymer-supported reagents affording the corresponding sulfonamides without further purification. Typically, an equimolar mixture of sulfonyl chlorides with the corresponding amines in the presence of polymer-bound morpholine were stirred overnight in DCM/DMF at room temperature using a Quest210 parallel synthesizer. Polymer-bound sulfonyl chloride and/or polymer-bound aminomethyl-PS were added to scavenge eventual excess of starting materials as detected by HPLC. The corresponding sulfonamides were obtained in high yield (60−98%) and purity (90−99%) (Scheme 7). At first, amines were selected based on their diversity and then in an increasingly focused way to culminate in 4-acyl- and alkylpiperidines, 4 arylpiperazines, and finally 4-benzotriazolopiperidines (Chart 2).

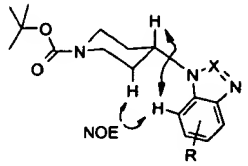
The latter led to the very promising benzotriazole subseries as highly active JNK inhibitors, which caused us to synthesize additional analogues. Because only two 4-benzotriazolopiperidines are commercially available, we elaborated a synthetic scheme to access such motifs (Scheme 8); the most straightforward way turned out to be a Mitsunobu-type reaction.[29] Benzotriazoles, benzimidazoles, or azabenzotriazoles were reacted with *N*-Boc-4-hydroxypiperidine in the presence of PPh_3 and DEAD in anhydrous THF, affording the corresponding

Scheme 8[a,b]



[a] Reagents: (a) *N*−Boc-4-hydroxypiperidine, DEAD, PPh_3, THF (20−80%), 3 h; (b) 20% TFA/DCM, 2 h (100%), (c) i. **2** or **29**, polymer-bound morpholine, DCM/DMF, ii polymer-bound aminomethylbenzene, polymer bound isocyanate. [b]Intermediates **44a−k** are deprotected to give intermediates **47a−k**, which are coupled ultimately to **2** or **29** to generate **50a−k**. Intermediates **45a−e** are deprotected to give intermediates **48a−e**, which are coupled to **2** or **29** to yield **51a−e**. Intermediates **46a−d** are deprotected to give intermediates **49a−d**, which are coupled ultimately to **2** or **29** to produce **52a−d**.

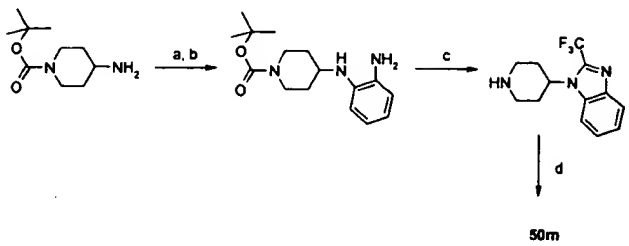
Chart 3. Assignment of Regioisomers of 1-(4-Piperidyl)-1-Boc-triazoles 44a–k, 45a–e, 46a–d Using 2D-NMR NOESY Experiments (cross-peaks between H^{7arom} with $H^{4piperdine}$ and $H^{3piperidine}$ were studied)



1-(4-piperidyl)-1-Boc-benzotriazoles, -benzimidazoles, or -azabenzotriazoles. In the case of unsubstituted benzotriazoles, two regioisomers could be expected, while for substituted benzotriazoles or heteroaromatic benzazoles, three different isomers were expected.

In all cases, all possible isomers were formed, but could be separated on gram scale using silica gel chromatography. The structures were determined using 2D-NMR experiments (Chart 3). Removal of Boc using standard techniques led to the novel building blocks of *N*-(4-piperidinyl)triazoles and imidazoles, which were ultimately coupled to sulfonyl chlorides **2** and **29**. Derivative **50m** could be accessed via a different route starting from 2-nitro-fluoro-benzene, which was coupled

Scheme 9[a]



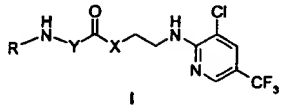
[a] Reagents: (a) 2-nitro-1-fluorobenzene, DMF, 80 °C, 15 h (78%); (b) H_2, Pd/C, EtOH, 3 h (75%); (c) TFA, DCM, 3 h (45%); (d) **2**, DIEA, DCM/DMF (60%).

to *N*-Boc-4-aminopiperidine in an SN_{Ar} type reaction. Reduction of the nitro-group led to the key intermediate, which was cyclized in one step to the desired 2-trifluoromethylbenzimidazole, after the Boc group had been removed (see Scheme 9).

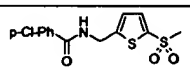
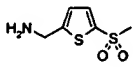
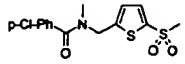
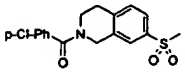
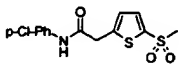
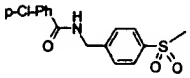
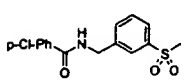
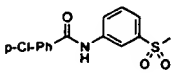
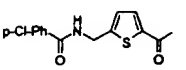
Results and Discussion

Already at the beginning of this work it was evident that the western benzoylaminomethyl moiety had an instrumental contribution for JNK activity, as pointed out in Table 2. Removal of this group led to a significant loss of activity (as exemplified by compound **32b**),

Table 2. In Vitro Activity of Key Compounds



I

Cpd	R	Y	X	IC50 (uM)[a] rJNK3	prep method[b]	anal.
I		NH	CH_2	0.30	A,B	a
16		CH_2	NH	1.1.	C	b
32b		NH	CH_2	>5	B	a
12		NH	CH_2	19	A	a
24		NH	CH_2	14	D	b
13		NH	CH_2	0.62	A	a
22		NH	CH_2	0.25	D	a
21		NH	CH_2	0.43	D	a
26		NH	CH_2	7	D	b
18		NH	CH_2	>5	C	a

[a] All values in triplicate. [b] Method A: prepared according to Scheme 2. Method B: prepared according to Scheme 6. Method C: prepared according to Scheme 3. Method D: prepared according to Scheme 4. [c] Analytical data. a: CHN, NMR, LC-MS; b: NMR, HPLC, LC-MS.

whereas modifications of the benzoylaminomethyl functionality offered only minor freedom to operate. Indeed, alkylation of the amide moiety resulted in compounds of reduced potency (**12** and **24**, Table 2), and only the inverse amide (cpd **13**, Table 2) retained activity to a certain degree. Replacement of the thiophene central core by a benzene ring (**21** and **22**, Table 2) was tolerated, and gave similar activity as hit **I**. However, removal of the methylene group (resulting in an aniline) lowered activity as shown by **26** (Table 2). At this point the decision was made to keep the central thiophene ring for further SAR evaluation, being a well-balanced compromise between ease of synthesis and ability to confer JNK activity.

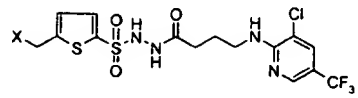
Additional exploration of the key binding elements in **I** revealed that the thiophene−sulfonamide linkage was crucial for activity, since loss of potency was observed when replacing it by an amide moiety (**18**, Table 2). Surprisingly, the effect was less pronounced when the SO_2 functionality was replaced by a methylene group, leading to only a 4-fold loss in activity. This may suggest that none of the oxygen atoms of the sulfonamide are directly implicated in binding, but the sulfonamide rather serves as a scaffold.

Finally, switching NH−NH−CO−CH_2 to NH−CH_2−CO−NH (**16**, Table 2) resulted in a 4-fold loss of inhibitory potency, suggesting that the eastern part of compound **I** could potentially be tailored.

Further profiling of the key western region of compound **I** established the preferred substitution of the aromatic ring (Table 3). The *p*-chlorine atom is not necessary for activity (**34a**) and can be replaced by many other substituents provided they keep the section flat (**34b−e**, **34g−i** and **34k,l**). However introduction of bulky substituents led to a drop in activity (**34f**, **34j**). Single meta substituents were acceptable (**34m−r**), but not meta disubstitution (**34w**), while ortho substituents tended to be deleterious (**34s,t** and **34v**) with the notable exception of OH (**34u**). We hypothesized here that the *o*-hydroxy group stabilizes the planarity of the benzamide moiety of these molecules.

We further demonstrated the critical importance of the aromatic amide group. Other aryl amides retain the activity (**35a−f**), but alkyl amides do not (**36a,f**) reconfirming that JNK inhibitory activity cannot be explained by a distinct hydrogen bond acceptor/donor interaction but rather by nonspecific hydrophobic (aromatic) interactions. Spacers between the aromatic ring and the amido group decrease the activity (**36b−e**). Furthermore, we found that the amide linkage could not be replaced by sulfonamide (**37**), urea (**38a−c**), or amine linkages (entry **39**) without loss of activity. For further optimization on the eastern end, we maintained the 4-chloro substitution, also because of its capability to prevent oxidative metabolism on the aromatic ring. We found that the (arylamino)butyrylhydrazino moiety on the eastern end was not strictly necessary for activity (Table 2) and that the isomeric glycine **16** was also active. However, when the γ-aminobutyric spacer was replaced by a more rigid linker, such as 4-carboxypiperidine, significant drop in activitity was observed (**33**). Alternatively, this moiety could be truncated altogether (**40a,b**, Table 4) or replaced by a piperazine or piperidine (**40c,d**) with a 3−10-fold loss of activity (Table 4).

Table 3. SAR Table of Western End Modifications

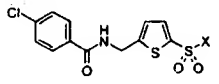


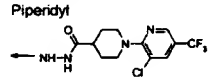
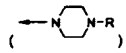
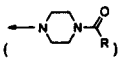
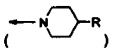
compd	R	IC50 (μM)[b] r-JNK3	IC50 (μM)[b] r-JNK2	prep method[a]	anal.[c]
	Benzamides, X = R-Ph-CONH				
34a	H	0.45	1.6	A	b
I	4-Cl	0.30	1.01	A	a
34b	4-F	0.33	1.8	A	b
34c	4-Br	0.46	1.5	A	b
34d	4-CH_3	0.52	0.79	A	b
34e	4-CH_2CH_3	0.64	1.6	A	b
34f	4-$C(CH_3)_3$	5.2	n.d.	A	a
34g	4-CF_3	0.51	1.58	A	a
34h	4-NO_2	0.98	1.7	A	b
34i	4-OCH_3	0.62	2	A	b
34j	4-$N(CH_3)_2$	>5	n.d.	A	b
34k	4-OH	0.62	7.5	A	b
34l	4-NH_2	0.54	3.5	A	b
34m	3-Cl	0.64	2.7	A	b
34n	3-Br	0.43	2.1	A	a
34o	3-OH	0.20	1.7	A	b
34p	3-OCH_3	0.21	3.6	A	b
34r	3-NO_2	0.26	1.2	A	a
34s	2-Cl	4.2	>5	A	b
34t	2-Br	3.0	>5	A	a
34u	2-OH	0.25	4.3	A	b
34v	2,6-dichloro	>5	n.d.	A	b
34w	3,5-dichloro	1.1	10	A	b
	Heteroaromatic Amides X = R-CONH				
35a	fur-2-yl	1.09	8.6	A	b
35b	pyridin-2-yl	0.38	n.d.	A	a
35c	pyridin-3-yl	0.68	8.7	A	a
35d	pyridin-4-yl	0.78	7.8	A	b
35e	3-pyridiyl-2-OH	0.40	n.d.	A	b
35f	3-pyridiyl-2-SH	0.23	n.d.	A	a
35g	3-pyridyl-2-NH_2	7.9	n.d.	A	a
	Alkyl Amides X = R-CONH				
36a	CH_3	>5	n.d.	A	a
36b	Ph-CH_2	>5	n.d.	A	b
36c	Ph-OCH_2	>5	n.d.	A	b
36d	Ph-CH_2CH_2	>5	n.d.	A	b
36e	Ph-CH=CH	6.5	n.d.	A	a
36f	cyclohexyl	>5	n.d.	A	b
	Sulfonamides, Ureas, and Amines X = R				
37	Ph-SO_2NH	>5	n.d.	B	a
38a	Ph-$CH_2NHCONH$	2.0	>5	C	b
38b	4-Cl−Ph-NHCONH	1.3	n.d.	C	a
38c	2-Cl−Ph-NHCONH	>5	n.d.	C	b
39	Ph-CH_2NH	> 10	n.d.	D	b

[a] Method A: prepared according to Scheme 6 route e. Method B: prepared according to Scheme 6 route f. Method C: prepared according to Scheme 6 route g. Method D: prepared according to Scheme 6 route h. [b] Purity = 87 ± 6%; all values in triplicate. [c] Analytical data. a: CHN, NMR, LC-MS; b: HPLC, LC-MS.

Activity could be regained by proper substitution of the piperazine or piperidine scaffold. In the piperazine series, *N*-aryl (**41a−d**) and *N*-alkyl (**41e−g**) derivatives proved to be poorly active, while *N*-acyl derivatives (**42b−e**) showed IC_{50}'s < 1 μM. In the piperidines series, substitution was implemented via −O− (**43a−c**), −NH− (**43d,e**), −CH− (**43f,g**), or −CO− (entry **43h**), linking groups. Compounds with IC_{50}'s ≤ 1 μM were found in all of the piperidine subseries, with the 1-hydroxybenzotriazolo- and the anilinopiperidines being the most interesting ones (**43c,d**). According to the results described above and the marked differences in activity depending on the carbocycle and its substitution pat-

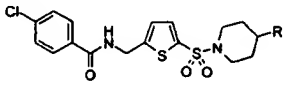
Table 4. SAR Table of Eastern End Modifications

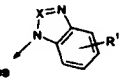


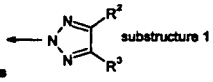
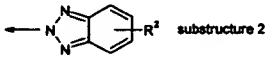
Cpd	X	IC50 [μM] JNK3	IC50 [μM] JNK2	Anal.[b]
40a	NH_2	3.5	n.d.	a
40b	$NH\text{-}NH_2$	2.3	n.d.	a
40c	Piperazinyl	1.1	0.6	a
40d	Piperidyl	2.1	n.d.	a
33		>5	>5	b
Piperazines	R (X)			
				
41a	Phenyl	>5	n.d.	a
41b	Pyrid-2-yl	2.3	2.4	a
41c	Pyrimid-2-yl	3.6	4.8	a
41d	3,5-di-OCH_3-phenyl	>5	n.d.	b
41e	Heptyl	>5	n.d.	b
41f	Benzyl	>5	n.d.	b
41g	Phenethyl	>5	n.d.	b
Piperazines-CO	R (X)			
				
42a	Phenyl	1.4	n.d.	b
42b	Hexyl	0.49	n.d.	a
42c	3,5-di-OCH_3-phenyl	0.64	1.60	b
42d	5-benzo[1,2,5]oxadiazole	0.51	1.03	a
42e	Phenethylenyl	0.63	0.98	b
Piperidines	R (X)			
				
43a	Hydroxy	1.4	n.d.	a
43b	Phenoxy	1.1	n.d.	b
43c	1-Hydroxybenzotriazole	0.78	n.d.	a
43d	Phenyl-NH	0.8	1.05	a
43e	(Pyrimid-2-yl)-NH	1.7	9.1	b
43f	Benzyl	>5	n.d.	b
43g	Phenethyl	1.3	n.d.	b
43h	Phenyl-CO-	1.3	n.d.	b

[a] All values in triplicate, standard deviations are below 15%. [b] Analytical data. a: CHN, NMR, LC-MS; b: NMR, LC-MS; typical purity >90%.

Table 5. SAR-table of Focused Eastern End Modifications

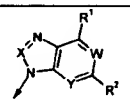


Cpd	X	R^1	IC50 [μM][a] JNK3	IC50 [μM][a] JNK2	Anal.[b]
1-benzazoles					
50a	N	H	0.15	0.65	a
50b	N	5-CF_3	0.46	1.30	a
50c	N	5-CO_2Me	0.19	1.4	a
50d	N	6-CO_2Me	0.16	0.31	b
50e	N	5-Cl	0.15	0.52	a.
50f	N	6-Cl	0.12	n.d.	b
50g	N	5-CO_2H	2.0	n.d.	b
50h	N	6-CO_2H	2.2	10	b
50i	CH	H	0.23	0.59	b
50j	CH	5-NO_2	0.31	n.d.	b
50k	CH	6-NO_2	0.18	n.d.	b
50l	CO	H	1.4	n.d.	b
50m	CCF_3	H	0.23	n.d.	b

	R^2	R^3			
2-benzazoles substructure 1  substructure 2 					
51a	-	-	2	>5	n.d.	a
51b	5-Cl		2	>5	n.d.	a
51c	5-CO_2Me		2	>5	n.d.	b
51d	6-CO_2Me		2	>5	n.d.	b
51e	H	H	1	2.68	n.d.	a

[a] All values in triplicate, standard deviations are below 15%. [b] Analytical data a: CHN, NMR, LC-MS; b: NMR, LC-MS; typical purity >90%.

Table 6. SAR Table of Focused Eastern End Modifications. Heteroaromatic Benzazoles

Cpd	X	Y	W	R^1	R^2	IC50 [μM][a] JNK3	IC50 [μM][a] JNK2	Anal.[b]
Heteroaromatic 1-benzazoles 								
52a	CH	N	CH	H	H	0.57	1.3	a
52b	CH	N	N	H	H	3.40	n.d.	b
52c	CH	N	N	NH_2	H	6.4	10	b
52d	CH	N	N	H	NH_2	2.0	n.d.	a

[a] All values in triplicate, standard deviations are below 15%. [b] Analytical data. a: CHN, NMR, LC-MS; b: NMR, LC-MS; typical purity >90%.

tern, it appears that a kink between the carbocyclic unit and an aromatic center is beneficial for JNK activity.

Compounds **41b**, **41c**, and **42a** were the first compounds synthesized in this SAR program that exhibited activity in a functional JNK3 cell assay of neuronal death, demonstrating that JNK inhibitors of this type block neuronal apoptosis in primary cultures of superior cervical ganglia (SCG) cells subjected to neuronal growth factor (NGF) deprivation. Compounds possessing a heteroatom in the kink or in the aromatic ring (e.g. **41b,c**) not only show improved inhibitory activity, but were also interesting at the cellular level. From a medicinal chemistry point of view, **43c** was considered as an attractive starting point for further investigations in this direction. Therefore, series of benzotriazolo-, benzimidazolo- (see Table 5) and azabenzotriazolopiperidines (see Table 6) were synthesized. The benzotriazole derivatives (Table 5, 1-benzazoles) confirmed our previous observations: compounds **50a**, **50c–f**, **50k** improved JNK3-activity by a factor of 2–3 as compared to **I**. Benzimidazoles were equally accepted as shown by **50i–k**, whereas benzimidazolones (e.g. **50l**) were less active. The fact that the benzene moiety points toward a hydrophobic pocket is underlined by the reduced activity of the 5-carboxy- and 6-carboxybenzotriazole derivatives (**50g–h**). Overall, the SAR is rather flat in this pocket, and a variety of different substituents appear to be equally acceptable. It is important to notice, however, that the isomeric 2-benzazole analogues (**51a–d**) rather disfavor JNK-inhibitory activity. Only the smallest unit, the 3,4*H*-triazole, retained some activity (**51e**).

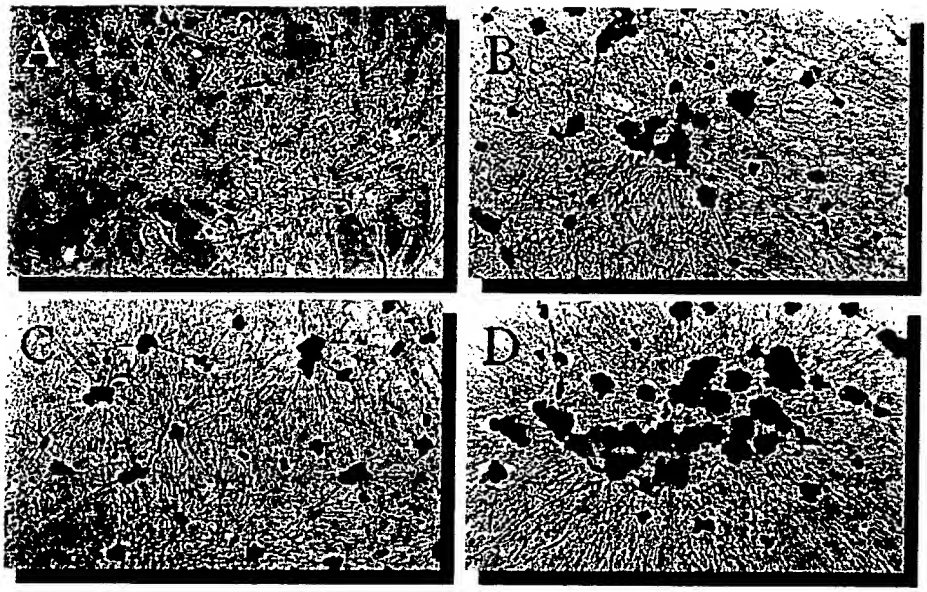


Figure 1. Effect of compound **50a** on neuronal survival. A: Control (−NGF). B: BAF treated, C: +NGF, D: **50a** @ 10 μM.

Table 7. Cell Activity of JNK Inhibitors in Neuronal Apoptosis Assay in SCG Cells

compd	concn (μM)	rescued neurons (%) 24 h	rescued neurons (%) 48 h	visual estimation of shape of neurons[a]
SB-239063[30]	10	18.1 ± 1.8 (2)	10.9 ± 1.8	−
I	10	<10	<10	
41c	10	22.9 ± 12.1 (4)	29.9 (1)	++
41b	10	43.3 ± 3.5 (2)	n.d.	
22	10	n.d.	2.1	
16	10	13.7 ± 0.3 (2)	33.4 (1)	+−
50a	10	27.3 ± 8.5 (10)	56.0 ± 9.7 (20)	+++
50a	3	22.9 (1)	25.2 ± 8.0 (2)	++
50a	1		2.2 ± 0.5 (2)	
34u	10	36.3 ± 19.6 (2)	63.8 ± 22 (5)	+++
34u	3	<10	<10	
42a	10	<10	<10	
42d	10	<10	<10	
40c	10	<10	<10	
50e	10	n.d.	67.3 ± 25.6 (2)	+++
50i	10	n.d.	36.1 ± 10.6 (2)	++

[a] Toxicity: visual estimation of shape of neurons; from − = bad shape (toxicity of cpd) to +++ perfect shape of neurons.

Having completed the SAR around this series, we wondered if the benzazole unit mimicked the adenine ring of ATP. Therefore, adenine and close analogues were selected and reacted according to Scheme 8. The fact that no gain in activity was obtained (Table 6) suggests that this is not the case. **52a** as the smallest aza analogue of **50i** displays a loss of activity by a factor of 2. Other analogues containing additional nitrogens (**52b**) and amino groups (**52c,d**) on the benzo moiety, which could potentially mimic adenine, are weak inhibitors in the micromolar range.

The most striking property of benzotriazole derivatives is a clearly improved cellular activity (Table 7). While **I** demonstrated activity below 5%, compounds **41c** and **41d** showed inhibitory activity in the range of 30%. However, the real breakthrough was achieved with **50a** and **50e** in particular, showing an excellent inhibition of neuronal cell death induced by in NGF deprived SCG cells at 10 μM (56.0% and 67%, respectively), which was still observed at 3 μM for **50a**. Full dose−response determination allowed to evaluate an IC_{50} value of 1.7 μM.

As shown in Figure 1, neurons are kept alive by treatment with **50a**, a significant protective effect comparable with that of the caspase inhibitor BAF (@ 100 μM). The antiapoptotic potential of this compound was further evaluated in a neuronal cell apoptosis model using human teratocarcinoma cells differentiated into neurons. In this model, apoptosis induced by serum deprivation was almost completely blocked by compound **50a** at 10μM, as well as by the caspase inhibitor BAF (100 μM).

Another important feature of this class of compounds is their excellent selectivity profile. **50a** was screened against 80 different Ser/Thr and Tyr-kinases, and did not significantly inhibit any of them at a concentration of 10 μM as shown in Table 8.

Nevertheless, the antiapoptotic potential of this new series of JNK inhibitor and especially compound **50a** did not correlate with in vivo potency in models of ischemia reperfusion injury due to a very poor biopharmaceutical profile. This was mainly due to very poor solubility of the compound (1 μg/mL in PBS) and to a PK profile characterized by low plasma exposure by both

Table 8. Selectivity Profile of Compound **50a** for 80 Protein Kinases[a]

protein kinase	activity (% of control)	protein kinase	activity (% of control)
JNK1α1(h)	20	MEK1(h)	98
JNK2α2(h)	16	MKK4(m)	112
JNK3(h)	6	MKK6(h)	106
Abl(m)	80	MKK7β(h)	121
AMPK(r)	90	MSK1(h)	86
Arg(m)	91	p70S6K(h)	77
Aurora-A(h)	79	PAK2(h)	88
Axl(h)	134	PDGFRα(h)	103
Blk(m)	78	PDGFRβ(h)	91
Bmx(h)	88	PDK1(h)	98
CaMKII(r)	93	PKA(h)	78
CaMKIV(h)	93	PKBα(h)	51
CDK1/cyclinB(h)	102	PKBβ(h)	118
CDK2/cyclinA(h)	99	PKBγ(h)	76
CDK2/cyclinE(h)	94	PKCα(h)-His	101
CDK3/cyclinE(h)	97	PKCβII(h)-His	88
CDK5/p35(h)	103	PKCγ(h)-His	79
CDK6/cyclinD3(h)	102	PKCδ(h)	95
CDK7/cyclinH/MAT1(h)	94	PKCε(h)	95
CHK1(h)	130	PKCη(h)	100
CHK2(h)	75	PKCι(h)	99
CK1(y)	85	PKCμ(h)	93
CK2(h)	99	PKCθ(h)	113
c-RAF(h)	93	PKD2(h)	95
CSK(h)	130	PRAK(h)	74
cSRC(h)	67	PRK2(h)	92
Fes(h)	134	ROCK-II(h)	101
FGFR3(h)	76	Rsk1(h)	93
Flt3(h)	78	Rsk2(h)	77
Fyn(h)	46	Rsk3(h)	97
IGF-1R(h)	108	SAPK2a(h)	77
IKKα(h)	92	SAPK2b(h)	100
IKKβ(h)	96	SAPK3(h)	106
IR(h)	103	SAPK4(h)	101
Lck(h)	85	SGK(h)	67
Lyn(h)	89	Syk(h)	75
MAPK1(h)	97	TrkB(h)	83
MAPK2(h)	106	Yes(h)	61
MAPKAP-K2(h)	74	ZAP-70(h)	121

[a] Protein kinases were assayed with 10 μM of **50a** in the presence of 10 μM of ATP.

iv (AUC_{rat} = 240 h·ng/mL @ 10 mg/kg), ip (AUC_{rat} = 85 h·ng/mL @ 10 mg/kg) and oral administration. It is therefore our next medicinal chemistry challenge to improve the 'druglike properties' of this series of JNK inhibitors.

Conclusion

We have identified a novel class of (benzoylaminomethyl)thiophene sulfonamide JNK inhibitors. Extensive SAR studies around the scaffold suggest that the western end and the central core appear to be essential features for inhibitory activity, allowing relatively little freedom to operate. In contrast, the eastern end of the molecule can accommodate a much larger range of substitutions, allowing for an improvement of potency in cell-based apoptosis assays. Taken together, these aspects have led to the identification of potent benzazole analogues. Compound **50a** as one representative of this subclass that inhibits the enzymatic activity of h-JNK3 with an IC_{50} value of 150nM and exhibits 4-fold selectivity against h-JNK2. **50a** displays an excellent selectivity profile toward a large range of receptors and enzymes, and more particularly, a stunning profile toward 80 Ser/Thr and Tyr-kinases. Moreover, **50a** shows excellent inhibition of neuronal cell death induced by NGF-deprived superior cervical ganglia cells (SCG cells) with an IC_{50} value of 1.7 μM. Its high enzymatic and cellular potency, associated with the high selectivity profile, allows **50a** to be a valuable tool to study JNK in biological systems.

Experimental Section

General Experimental Methods. Procedures. All chemicals were purchased from Fluka-Aldrich, Buchs (CH), unless otherwise stated. All polymer-bound reagents were purchased from Argonaut Technologies. Parallel syntheses were carried out in a Quest210 parallel synthesizer from Argonaut Technologies. Parallel evaporations were performed in a HT-4 Atlas Evaporator from GeneVac. Melting points were measured with an apparatus Büchi Melting Point B-545 and were uncorrected. NMR spectra were recorded on a Brucker DPX-300 MHz spectrometer. Data were reported as follows: chemical shift in ppm using either residual DMSO (2.49 ppm) or $CHCl_3$ (7.19 ppm) as internal standards on the δ scale, multiplicity (s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet), coupling constants (s) in hertz, and integration. MS data provided were obtained using a mass spectrometer Perkin-Elmer API 150 EX (APCI). The analytical HPLC was performed using a HPLC column Waters Symmetry C8 50 × 4.6 mm, conditions: a- MeCN/H_2O 0.09% TFA, 0 to 100% (10 min); b- MeCN/H_2O 0.09% TFA, 0 to 100% (20 min); c- MeCN/H_2O 0.09% TFA, 5 to 100% (10 min), max plot 230–400 nm; d- MeCN/H_2O, 5 to 100% (10 min), max plot 230–400 nm. Elemental analyses were performed on an Erba Science 11108 CHN analyzer.

4-Chloro-*N*-thiophen-2-ylmethylbenzamide (1). A solution of 4-chlorobenzoyl chloride (14.52 mL, 114 mmol) in 50 mL of dry DCM was added over 30 min to a stirred solution of 2-aminomethylthiophene (14.1 mL, 137 mmol) and i-Pr_2NEt (43.0 mL, 251 mmol) in DCM (200 mL) at 0 °C. A white solid was formed, and the reaction was allowed to warm to room temperature over 1 h. The mixture was diluted with 200 mL of DCM, washed twice with HCl aq (0.1 N), and dried over $MgSO_4$. Evaporation gave 28 g (98%) of the title benzamide as a white solid: mp 153–54 °C, ^1H NMR ($CDCl_3$) δ 7.90 (d, J = 8.67 Hz, 2H), 7.58 (d, J = 8.67 Hz, 2H), 7.44 (dd, J = 3.77, 1.13 Hz, 1H), 7.22 (d, J = 5.27 Hz, 1H), 7.16 (dd, J = 3.39, 5.27 Hz, 1H), 6.62 (br d, 1H), 4.98 (d, J = 5.65 Hz, 2H).

5-({[1-(4-Chlorophenyl)methanoyl]-amino}methyl)-thiophene-2-sulfonyl Chloride (2). A solution of **1** (10 g, 40 mmol) in DCM (500 mL) was treated with a solution of chlorosulfonic acid (20.1 mL, 198 mmol) in DCM (80 mL) at −80 °C. The reaction mixture was allowed to reach rt over 5 h. The mixture was poured on ice and quickly extracted with DCM. The organic layer was dried over $MgSO_4$, and the solvent was evaporated to dryness to yield 8.8 g (63%) of **2** as a white powder which was used without further purification: mp 133–35 °C, ^1H NMR (DMSO-d_6) δ 9.21 (t, J = 6.4 Hz, 1H), 7.87 (d, J = 8.7 Hz, 2H), 7.53 (d, J = 8.7 Hz, 2H), 6.91 (d, J = 3.4 Hz, 1H), 6.77 (d, J = 3.4 Hz, 1H), 4.53 (d, J = 3.8 Hz, 2H).

4-Chloro-*N*-methyl-*N*-thiophen-2-ylmethylbenzamide (3). To a slurry of 318 mg of NaH (60% purity, 7.94 mmol) in dry THF (50 mL) was added **1** (1 g, 3.97 mmol) in THF at 0 °C under N_2. The slurry was stirred for 15 min at rt prior to the addition of 1 mL of MeI (15.9 mmol). The reaction was stirred for 1 h, water was slowly added, and the reaction mixture was evaporated to dryness. EtOAc was added, and the organic layer was washed with water, dried over $MgSO_4$, and evaporated to dryness to yield 1 g (98%) of **3** as a transparent oil. ^1H NMR (DMSO-d_6 60°C) δ 7.49 (d, J = 8.66 Hz, 2H), 7.45–7.39 (m, 3H), 7.03 (br s, 1H), 7.01–6.90 (m, 1H), 4.72 (br s, 2H), 2.88 (s, 3H), MS m/z 266.5 (M + H).

5-{[(4-chlorobenzoyl)methyl-amino]methyl}thiophene-2-sulfonyl Chloride (4). **4** was synthesized according to the chlorosulfonylation procedure of **2**. Isolated yield: 650 mg (48%). ^1H NMR (DMSO-d_6) δ 7.86 (d, J = 8.7 Hz, 2H), 7.51 (d, J = 8.7 Hz, 2H), 6.90 (d, J = 3.4 Hz, 1H), 6.78 (d, J = 3.4 Hz, 1H), 4.76 (s, 2H), 2.95 (s, 3H).

***N*-(4-Chlorophenyl)-2-thiophen-2-ylacetamide (5).** **5** was synthesized starting from 5 g of thiophen-2-yl-acetyl chloride according to the synthesis of **1**. Isolated yield: 6.7 g (86%) of a beige solid. ^{1}H NMR ($CDCl_3$) δ 7.65 (d, J = 9.0 Hz, 2H), 7.58 (dd, J = 1.5 Hz, 4.9 Hz, 1H), 7.51 (d, J = 9.0 Hz, 2H), 7.35−7.25 (m, 2H), 4.20 (s, 2H), MS *m/z* 252.0 (M + H); 250.0 (M − H).

5-[(4-Chlorophenylcarbamoyl)methyl]thiophene-2-sulfonyl Chloride (6). **6** was synthesized according to the chlorosulfonylation procedure for **2**. Isolated yield: 330 mg (47%) of white crystals. ^{1}H NMR ($CDCl_3$) δ 7.76 (d, J = 3.7 Hz, 1H), 7.44 (d, J = 8.6 Hz, 2H), 7.29 (d, J = 8.6 Hz, 2H), 7.24 (s, 1H), 7.0 (d, J = 3.7 Hz, 1H), 3.98 (s, 2H).

4-(3-Chloro-5-trifluoromethylpyridin-2-ylamino)butyric Acid Methyl Ester (10). A mixture of γ-aminobutyric acid (8.18 g, 79.3 mmol), 2,3-dichloro-5-(trifluoromethyl)-pyridine (11.0 mL, 79.3 mmol), triethylamine (27.6 mL, 198.3 mmol), and methanol (270 mL) was heated at 100−04 °C in a Parr autoclave (450 mL vessel) with mechanical agitation for 3 h. Evaporation, addition of DCM (200 mL), and filtration removed the unreacted, insoluble γ-aminobutyric acid (2.5 g). Evaporation, addition of *t*-BuOMe (200 mL), and filtration removed most of the Et_3N·HCl salt (4.4 g). The ether solution was filtered through a silica gel plug and concentrated to afford the crude N-substituted γ-aminobutyric acid **9** as its triethylammonium salt. The crude was esterified to the corresponding methyl γ-aminobutyrate **10** by heating to reflux for 1.5 h in methanolic H_2SO_4 (1.9 M H_2SO_4 in MeOH, 50 mL). Concentration, addition of EtOAc (100 mL) and cyclohexane (100 mL), washing ($NaHCO_3$ sat.; H_2O; brine), drying (Na_2SO_4), and evaporation afforded 13.8 g (59%) **10** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 8.18 (d, J = 0.9 Hz, 1H), 7.53 (d, J = 2.2 Hz, 1H), 5.54−5.42 (br. t, J = 6 Hz, 1H), 3.61 (s, 3H), 3.51 (q, J = 6.8 Hz, 2H), 2.35 (t, J = 7.2 Hz, 2H), 1.92 (quint, J = 7.0 Hz, 2H).

4-(3-Chloro-5-trifluoromethylpyridin-2-ylamino)butyric Acid Hydrazide (11). A solution of **10** (5.61 g, 19.0 mmol) in 80% aqueous hydrazine (7 mL) and MeOH (14 mL) was heated to reflux for 2 h. The reaction mixture was diluted with EtOAc (250 mL). The unreacted hydrazine was extracted with a minimum amount of water. The organic layer was dried (Na_2SO_4), concentrated to 50 mL, and poured into a crystallizer containing 150 mL of cyclohexane. The desired hydrazide rapidly crystallized, and filtration after 2 h afforded 4.24 g (76%) of **11** as pale yellow needles: ^{1}H NMR (DMSO-d_6) δ 8.96 (br s, 1H), 8.32 (br s, 1H), 7.94 (d, J = 2.1 Hz, 1H), 7.25 (t, J = 5.5 Hz, 1H), 4.51 (s, 2H), 3.40 (q, J = 6.6 Hz, 2H), 2.07 (t, J = 7.6 Hz, 2H), 1.88 (quint, J = 7.2, 2H); MS *m/z* 297 (M + H).

4-Chloro-*N*-[(5-{[2-(4-{[3-chloro-5-(trifluoromethyl)pyridin-2-yl]amino}butanoyl)hydrazino]sulfonyl}-2-thienyl)-methyl]benzamide (I). A solution of **2** (211 mg, 0.60 mmol), the acyl hydrazide **11** (179 mg, 0.60 mmol), and pyridine (71 mg, 0.90 mmol) in $CHCl_3$ (10 mL) was heated to reflux for 3 h and cooled to rt. The precipitate was collected by filtration and washed with $CHCl_3$ to give 321 mg (88%) of the title compound **I** as a white powder. ^{1}H NMR (DMSO-d_6) δ 10.07 (d, J = 3.2 Hz, 1H), 9.88 (s, 1H), 9.25 (t, J = 5.3 Hz, 1H), 8.32 (s, 1H), 7.95 (d, J = 2.0 Hz, 1H), 7.78 (d, J = 8.6 Hz, 2H), 7.54 (d, J = 8.6 Hz, 2H), 7.42 (d, J = 3.8 Hz, 1H), 7.25 (br. t, J = 5.5 Hz, 1H), 7.03 (d, J = 3.8 Hz, 1H), 4.61 (d, J = 5.9 Hz, 2H), 3.32 (buried q, 2H), 2.04 (t, J = 7.4 Hz, 2H), 1.65 (quint, J = 7.1 Hz, 2H). MS *m/z* 610.2(M + H); 608.1 (M − H); Anal. ($C_{22}H_{20}Cl_2F_3N_5O_4S_2$): C, H, N.

4-Chloro-*N*-[(5-{[2-(4-{[3-chloro-5-(trifluoromethyl)pyridin-2-yl]amino}butanoyl)hydrazino]sulfonyl}-2-thienyl)-methyl]-*N*-methylbenzamide (12). **12** was synthesized using **4** as sulfonyl chloride according to the synthesis of **I**. Isolated yield after silica gel chromatography (DCM/MeOH 20:1): 20 mg (83%) of a white solid. ^{1}H NMR ($CDCl_3$) δ 9.98 (br s, 1H), 8.33 (s, 1H), 7.62 (d, J = 1.9 Hz, 1H), 7.53 (d, J = 3.7 Hz, 1H), 7.36 (s, 4H), 7.24 (s, 1H), 6.99 (br s, 1H), 5.70 (t, J = 5.8 Hz, 1H), 4.76 (br s, 2H), 3.48 (q, J = 6.5 Hz, 2H), 2.95 (br s, 3H), 2.23 (t, J = 6.2 Hz, 2H), 1.85 (quin, J = 6.6 Hz, 2H), MS *m/z* 624.9 (M + H); 622.2 (M − H); Anal. ($C_{23}H_{22}Cl_2F_3N_5O_4S_2$): C, H, N.

***N*-(4-Chlorophenyl)-2-(5-{[2-(4-{[3-chloro-5-(trifluoromethyl)pyridin-2-yl]amino}butanoyl)hydrazino]sulfonyl}-2-thienyl)acetamide (13).** **13** was synthesized using **6** as sulfonyl chloride according to the synthesis of **I**. Isolated yield after silica gel chromatography: 40%. ^{1}H NMR (DMSO-d_6) δ 10.3 (s, 1H), 10.0 (d, J = 2.6 Hz, 1H), 9.86 (d, J = 3.4 Hz, 1H), 8.29 (s, 1H), 7.91 (d, J = 2.3 Hz, 1H), 7.59 (d, J = 9.0 Hz, 2H), 7.44 (d, J = 3.7 Hz, 1H), 7.33 (d, J = 9.0 Hz, 2H), 7.24 (t, J = 5.4 Hz, 1H), 7.00 (d, J = 3.7 Hz, 1H), 4.06 (s, 2H), 3.4−3.3 (m, 2H), 2.05 (t, J = 7.5 Hz, 2H), 1.66 (quint, J = 6.9 Hz, 2H), MS *m/z* 610.1 (M + H); 608.1 (M − H); Anal. ($C_{22}H_{20}Cl_2F_3N_5O_4S_2$): C, H, N.

{5-[(4-Chlorobenzoylamino)methyl]thiophene-2-sulfonylamino}acetic Acid (14). Glycine *tert*-butyl ester (263 mg, 1.57 mmol) and DIEA (537 μL) were dissolved in 20 mL of DCM. To this solution was added **2** (500 mg, 1.43 mmol) in 10 mL of DMF. The reaction was stirred overnight. After aqueous workup, the corresponding *tert*-butyl ester was isolated as a white solid (400 mg, 63%) and deprotected without any further purification using DCM/TFA 1:1. The reaction mixture was stirred for 1 h. The solvents were evaporated to dryness to yield **14** as a white solid (330 mg, 95%). ^{1}H NMR (DMSO-d_6) δ 9.34 (t, J = 5.8 Hz, 1H), 8.20 (t, J = 6.0 Hz, 1H), 7.88 (d, J = 8.7 Hz, 2H), 7.55 (d, J = 8.7 Hz, 2H), 7.42 (d, J = 3.7 Hz, 1H), 7.04 (d, J = 3.7 Hz, 1H), 4.62 (d, J = 5.6 Hz, 2H), 3.58 (d, J = 6.0 Hz, 2H), MS *m/z* 387.6 (M − H).

4-Chloro-*N*-[5-({[2-(3-chloro-5-trifluoromethyl-pyridin-2-ylamino)ethylcarbamoyl]methyl}sulfamoyl)thiophen-2-ylmethyl]benzamide (16). The intermediate **14** (320 mg, 0.82 mmol), DIEA (2.46 mmol), and PyBOP (860 mg, 1.7 mmol) were dissolved in 30 mL of DCM. To this solution was added the amine **15** (296 mg, 1.25 mmol) in 20 mL of DCM. The reaction was stirred for 15 h, the solvent was evaporated to dryness, and the crude material was purified on silica gel (cyclohexane/EtOAc 1:1) affording 480 mg (95%) of **16** as a white solid. ^{1}H NMR (DMSO-d_6) δ 9.34 (t, J = 5.8 Hz, 1H), 8.31 (s, 1H), 8.09 (q, J = 6.5 Hz, 2H), 7.95 (d, J = 2.3 Hz, 1H), 7.87 (d, J = 8.7 Hz, 2H), 7.54 (d, J = 8.7 Hz, 2H), 7.42 (d, J = 3.7 Hz, 1H), 7.28 (t, J = 5.5 Hz, 1H), 7.05 (d, J = 3.7 Hz, 1H), 4.62 (d, J = 5.6 Hz, 2H), 3.56−3.07 (m, 6H). MS *m/z* 610.3 (M + H); 608.1 (M − H); Anal. ($C_{22}H_{20}Cl_2F_3N_5O_4S_2$): C, H, N.

5-Aminomethylthiophene-2-carboxylic Acid Methyl Ester (17). 5-Methylthiophene-2-carboxylic acid (5 g, 35 mmol) was refluxed in H_2SO_4 in MeOH (2 M) for 6 h. The reaction was neutralized with NaOH (10 N) at 0 °C, and the methyl ester was extracted with DCM affording 4.85 g (89%) of 5-methylthiophene-2-carboxylic acid methyl ester. Without further purification, the methyl ester (4.8 g, 31 mmol) was refluxed in CCl_4 in the presence of NBS (6 g, 34 mmol) and benzoyl peroxide (242 mg, 0.03 equiv) for 20 h. The reaction is cooled to 0 °C and filtered. The filtrate is concentrated to dryness affording an oily orange liquid representing 5-bromomethylthiophene-2-carboxylic acid methyl ester (7 g, 98%). NMR shows 80% of the monobromo derivative, which was used for the next step without further purification. Seven grams (24 mmol, 80%) of the obtained mixture was heated at 70 °C in DMF in the presence of 2.2 g (33 mmol) of NaN_3 for 3 h. EtOAc was added, and the organic layer was washed with brine several times affording 5-azidomethylthiophene-2-carboxylic acid methyl ester (5.5 g). Mass spectrometry indicated the absence of the bromo derivative. The azido intermediate (5.5 g, 80% purity) was reduced in the presence of Pd/C with H_2 at 2 bar in 10% HCl (2 M) in EtOH for 20 h. The acidic solution was then washed with DCM and further basified to pH 9.5. The aqueous basic solution was then extracted several times with DCM to yield 2 g of **17** (50%) as a yellow liquid, which spontaneously crystallized after solvent evaporation. ^{1}H NMR ($CDCl_3$) δ 7.64 (d, J = 3.9 Hz, 1H), 6.89 (d, J = 3.9 Hz, 1H), 4.05 (s, 2H), 3.84 (s, 3H), 1.54 (s, 2H), MS *m/z* 171 (M + H).

4-Chloro-*N*-(5-{*N'*-[4-(3-chloro-5-trifluoromethylpyridin-2-ylamino)butyryl]hydrazinocarbonyl}thiophen-2-ylmethyl)benzamide (18). Intermediate **17** (500 mg, 2.92 mmol) was capped with 4-chlorobenzoyl chloride (355 μL, 2.8 mmol) in the presence of DIEA (750 μL, 4.4 mmol) in DCM. The reaction was stirred for 2 h at rt. Aqueous workup produced 890 mg (90%) of the corresponding methyl ester, which was saponified directly. The methyl ester (770 mg, 2.48 mmol) was dissolved in 18 mL of THF/water (5:1), $LiOH \cdot H_2O$ (208 mg, 4.98 mmol) was added, and the reaction was heated at 60 °C for 5 h. pH was decreased to 1, and the precipitate was filtered off and washed with a small amount of water. The corresponding acid was isolated as a white powder (725 mg, 98%) (NMR indicated absence of the methyl group). The acid (30 mg, 0.1 mmol) was coupled directly to **11** (33 mg, 0.11 mmol) using DCI (14 mg, 0.11 mmol) and HOBt (15 mg, 0.11 mmol) as coupling reagents in DCM/DMF 1:1. The reaction mixture was stirred for 3 h at rt, while **18** precipitated out as a colorless solid (34 mg, 60%). ^1H NMR (DMSO-d_6) δ 10.2 (s, 1H), 9.84 (s, 1H), 9.29 (t, J = 5.8 Hz, 1H), 8.32 (s, 1H), 7.94 (d, J = 2.3 Hz, 1H), 7.88 (d, J = 8.7 Hz, 2H), 7.64 (d, J = 3.7 Hz, 1H), 7.54 (d, J = 8.7 Hz, 2H), 7.36 (t, J = 5.5 Hz, 1H), 7.04 (d, J = 3.7 Hz, 1H), 4.61 (d, J = 5.7 Hz, 2H), 3.45 (q, J = 6.4 Hz, 2H), 2.20 (t, J = 7.5 Hz, 2H), 1.81 (quint, J = 7.3 Hz, 2H), MS *m/z* 574.3 (M + H); 572.1 (M − H); Anal. ($C_{23}H_{20}Cl_2F_3N_5O_2S$): C, H, N.

***N'*-{[3-(Aminomethyl)phenyl]sulfonyl}-4-{[3-chloro-5-(trifluoromethyl)pyridin-2-yl]amino}butanohydrazide (19).** **19** was synthesized from 3-cyanobenzenesulfonyl chloride (372 mg, 1.86 mmol) according to the synthesis of **I**. Isolated yield of the corresponding nitrile after silica gel chromatography: 750 mg (87%). The nitrile (360 mg, 0.78 mmol) was reduced using $LiAlH_4$ (1.9 mL (1 M in THF), 1.95 mmol) in anhydrous THF at rt. The reaction mixture was stirred for 30 min at rt before quenching with H_2O. Aqueous workup afforded 363 mg (97%) of **19**. ^1H NMR (DMSO-d_6) δ 8.30 (s, 1H), 7.92 (d, J = 2.3 Hz, 1H), 7.78 (s, 1H), 7.64 (d, J = 7.9 Hz, 1H), 7.55 (d, J = 7.9 Hz, 1H), 7.44 (t, J = 7.7 Hz, 1H), 7.27 (t, J = 5.8 Hz, 1H), 3.8 (s, 2H), 3.30 (q, J = 6.5 Hz, 2H), 2.01 (t, J = 7.3 Hz, 2H), 1.61 (quint, J = 7.2 Hz, 2H), MS *m/z* 466.2 (M + H); 464.1 (M − H).

***N'*-{[4-(Aminomethyl)phenyl]sulfonyl}-4-{[3-chloro-5-(trifluoromethyl)pyridin-2-yl]amino}butanohydrazide (20).** **20** was synthesized according to the synthesis of **19** to yield 260 mg (67%) of **20**. ^1H NMR (CD_3OD) δ 8.22 (s, 1H), 7.94 (d, J = 8.3 Hz, 2H), 7.81 (d, J = 1.9 Hz, 1H), 7.66 (d, J = 8.3 Hz, 2H), 4.21 (s, 2H), 3.44 (t, J = 6.9 Hz, 2H), 2.16 (t, J = 7.3 Hz, 2H), 1.78 (quint, J = 7.2 Hz, 2H), MS *m/z* 466.2 (M + H); 464.1 (M − H).

4-Chloro-*N*-(3-{[2-(4-{[3-chloro-5-(trifluoromethyl)pyridin-2-yl]amino}butanoyl)hydrazino]sulfonyl}benzyl)-benzamide (21). To a solution of **20** (15.7 mg, 34 μmol) in DCM/pyridine 30:1 was added 4-chlorobenzoyl chloride (0.7 equiv) at rt. The reaction mixture was stirred for 2 h at rt. The crude product was filtered through silica gel affording pure **21** (15.9 mg, 78%) as a colorless solid. ^1H NMR (DMSO-d_6) δ 10.5 (s, 1H), 9.98 (s, 1H), 9.79 (br s, 1H), 8.28 (br s, 1H), 8.03 (d, J = 8.7 Hz, 2H), 7.90 (d, J = 1.9 Hz, 1H), 7.64 (d, J = 8.7 Hz, 2H), 7.52−73.4 (m, 3H), 7.27−7.13 (m, 1H), 1.99 (t, J = 6.4 Hz, 2H), 1.62 (quint, J = 6.9 Hz, 2H), 1.22 (s, 2H), MS *m/z* 604.1 (M + H); 602.0 (M − H); Anal. ($C_{24}H_{22}Cl_2F_3N_5O_4S$): C, H, N.

4-Chloro-*N*-(4-{[2-(4-{[3-chloro-5-(trifluoromethyl)pyridin-2-yl]amino}butanoyl)hydrazino]sulfonyl}benzyl)-benzamide (22). **22** was synthesized according to synthesis of **21**. Isolated yield: 15 mg (73%). ^1H NMR (DMSO-d_6) δ 9.95 (d, J = 3.0 Hz, 1H), 9.73 (d, J = 3.4 Hz, 1H), 9.20 (t, J = 5.6 Hz, 1H), 8.29 (s, 1H), 7.92 (d, J = 1.9 Hz, 1H), 7.89 (d, J = 8.7 Hz, 2H), 7.74 (d, J = 8.7 Hz, 2H), 7.53 (d, J = 8.7 Hz, 2H), 7.44 (d, J = 8.7 Hz, 2H), 7.23 (t, J = 5.6 Hz, 1H), 4.51 (d, J = 5.6 Hz, 2H), 3.28 (q, J = 6.4 Hz, 2H), 1.99 (t, J = 6.9 Hz, 2H), 1.61 (quint, J = 6.9 Hz, 2H), MS *m/z* 604.1 (M + H); 602.0 (M − H); Anal. ($C_{24}H_{22}Cl_2F_3N_5O_4S$): C, H, N.

4-{[3-Chloro-5-(trifluoromethyl)pyridin-2-yl]amino}-*N'*-(1,2,3,4-tetrahydroisoquinolin-7-ylsulfonyl)butanohydrazide (23). **23** was produced following the synthetic procedure for **I** using 2-acetyl-1,2,3,4-tetrahydroisoquinoline-7-sulfonyl chloride (357 mg, 1.09 mmol). The corresponding trifluoroacetamide of **23** was isolated in quantitative yield and was directly deprotected using 1 mL of sat. K_2CO_3 in 10 mL of MeOH at 50 °C for 1 h. Filtration over silica gel using DCM/MeOH/NH_4OH 80:20:5 as eluents produced 280 mg (94%) of **23** as a white powder. ^1H NMR (DMSO-d_6) δ 9.99 (br s, 1H), 8.30 (s, 1H), 7.93 (s, 1H), 7.60 (s, 1H), 7.57 (s, 1H), 7.29 (d, J = 7.2 Hz, 2H), 4.10 (s, 2H), 3.30 (q, J = 6.3 Hz, 2H), 3.16 (t, J = 5.8 Hz, 2H), 2.89 (t, J = 5.6 Hz, 2H), 2.01 (t, J = 7.7 Hz, 2H), 1.62 (quint, J = 7.2 Hz, 2H), MS *m/z* 492.1 (M + H); 490.5 (M − H).

***N'*-{[2-(4-Chlorobenzoyl)-1,2,3,4-tetrahydroisoquinolin-7-yl]sulfonyl}-4-{[3-chloro-5-(trifluoromethyl)pyridin-2-yl]amino}butanohydrazide (24).** Compound **24** was synthesized following the procedure for the synthesis of **21**. Isolated yield: 19.7 mg (51%). ^1H NMR (DMSO-d_6) δ 9.99 (br s, 1H), 8.30 (s, 1H), 7.93 (s, 1H), 7.88 (d, J = 8.7 Hz, 2H), 7.60 (s, 1H), 7.55 (d, J = 8.7 Hz, 2H), 7.57 (s, 1H), 7.29 (d, J = 7.2 Hz, 2H), 4.10 (s, 2H), 3.30 (q, J = 6.3 Hz, 2H), 3.16 (t, J = 5.8 Hz, 2H), 2.89 (t, J = 5.6 Hz, 2H), 2.01 (t, J = 7.7 Hz, 2H), 1.62 (quint, J = 7.2 Hz, 2H), MS *m/z* 630.3 (M + H); 628.5 (M − H); Anal. ($C_{26}H_{24}Cl_2F_3N_5O_4S$): C, H, N.

***N'*-[(3-Aminophenyl)sulfonyl]-4-{[3-chloro-5-(trifluoromethyl)pyridin-2-yl]amino}butanohydrazide (25).** To a solution of 3-nitrobenzenesulfonyl chloride (89 mg, 0.40 mmol) and DMAP (72 mg, 0.59 mmol) in DMF was added **11** (120 mg, 0.40 mmol). The reaction was stirred at rt for 5 h. Aqueous workup produced a brown solid, which was purified on silica gel using $CHCl_3$/EtOAc 4:1 to 1:1. 92 mg (47%) of the corresponding nitro intermediate were isolated as a white solid. The nitro intermediate (89 mg, 0.185 mmol) was dissolved in 2 mL of DMF followed by the addition of 52 mg (0.23 mmol) of $SnCl_2 \cdot 2H_2O$. The reaction was stirred overnight and was driven to completion by adding additional 53 mg of the reducing agent. The crude product was filtered over silica gel using EtOAc/MeOH 98:2 as eluent to yield 63 mg (75%) of **25** as a pale yellow solid. MS *m/z* 452.8 (M + H); 450.7 (M − H).

4-Chloro-*N*-(3-{[2-(4-{[3-chloro-5-(trifluoromethyl)pyridin-2-yl]amino}butanoyl)hydrazino]sulfonyl}phenyl)-benzamide (26). Compound **26** was synthesized using the protocol of **I** and could be isolated as a pale yellow powder after silica gel chromatography in 35% yield. MS *m/z* 591.4 (M + H); 589.5 (M − H); Anal. ($C_{23}H_{20}Cl_2F_3N_5O_4S$): C, H, N.

5-[(3-Methoxybenzoylamino)methyl]thiophene-2-sulfonyl Chloride (29). To a solution of 2-aminomethylthiophene (10.6 mL, 103 mmol) and pyridine (9.1 mL, 104 mmol) in 100 mL of chloroform was added at 0 °C a solution of 3-methoxybenzoyl chloride (19.2 g, 103 mmol) in DCM. The reaction mixture was allowed to warm to rt during 1 h and stirred for additional 3 h. Water was added while 3-methoxy-*N*-(thien-2-ylmethyl)benzamide **27** (10.1 g) precipitated. The solid was filtered off and washed with water. The remaining organic layer was washed with brine, dried over $MgSO_4$, and evaporated to dryness to afford additional 15.2 g of **27**. The overall yield was 25.3 g (99.9%). **27** was used for the next step without further purification. Chlorosulfonic acid (5.62 mL, 84 mmol) was dissolved in 20 mL DCM and added to a solution of **27** (11.0 g, 42 mmol) in 100 mL of DCM under vigorous stirring. A gummy solid was formed, and the reaction mixture was stirred for 3 h. The reaction was quenched with ice, and ice cold $NaHCO_3$ solution was added to reach pH 8.5. The aqueous layer was washed twice with DCM. Tetrabutylammonium hydroxide (40% in water) (32 mL, 50 mmol) was added to the aqueous layer, while a solid was formed. The precipitate was extracted with DCM, and the aqueous layer was washed 3× with DCM. The combined organic layers were dried over $MgSO_4$ and evaporated to dryness to afford a slightly colored foam of tetrabutylammonium 5-{[(3-methoxybenzoyl)-amino]-methyl}thiophene-2-sulfonate **28** (24 g, 97%). NMR spectra indicated pure compound, which was used for the following

chlorination step. To a solution of **28** (2.0 g, 3.4 mmol) in 50 mL of DCM was added triphosgene (800 mg, 2.7 mmol, 2.3 equiv), dissolved in 10 mL of DCM. To this reaction mixture was added DMF (0.1 mL, 1.4 mmol) dropwise during 10 min, while gas evolution could be observed. The reaction mixture was stirred for 3 h, and the crude product was directly filtered through silica gel using EtOAc/hexane 1:2 as eluent. An orange solid could be isolated which was further recrystallized from cyclohexane/DCM. **29** (730 mg, 60%) was obtained as colorless needles. ^{1}H NMR ($CDCl_3$) δ 8.83 (t, J = 1.5 Hz, 1H), 8.35 (t, J = 7.5 Hz, 1H), 7.76 (t, J = 4.1 Hz, 1H), 7.70–7.58 (m, 3H), 7.52–7.40 (m, 2H), 7.05 (t, J = 3.8 Hz, 1H).

Diallylthiophen-2-ylmethylamine (30). Allyl bromide (55 mL, 65.4 mmol) was added to a solution of 2-aminomethylthiophene (24 mL, 23.3 mmol) and *i*-Pr_2NEt (120 mL, 70.1 mmol) in DCM (270 mL). The moderately exothermic reaction spontaneously reached the reflux temperature after 1 h. The reaction was cooled by means of an ice bath and stirred for 14 h at rt whereupon a precipitate appeared. The organic layer was concentrated, and the precipitates were filtered off. The EtOAc solution was filtered over SiO_2 and concentrated to give 36.1 g (80%) of **30** as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.25 (br d, J = 5.9 Hz, 1H), 6.98 (br dd, J = 5.1, 2.8 Hz, 1H), 6.94–6.92 (m, 1H), 5.99–5.86 (m, 2H), 5.29–5.18 (m, 4H), 3.85 (s, 2H), 3.16 (dd, J = 6.3, 0.9 Hz, 4H).), MS *m/z* 194.2 (M + H).

5-Diallylaminomethylthiophene-2-sulfonyl Chloride (31). A solution of the allyl-protected thiophene **30** (6.2 g, 32.1 mmol) in Et_2O was cooled to − 70 °C by means of an acetone/dry ice bath. A solution of *t*-BuLi in pentane (21.38 mL, 1.5 M, 32.1 mmol) was added over 2 min whereupon the internal temperature rose to −50 °C and the mixture turned orange. After 10 min, SO_2 gas was bubbled for 2 min, which led to the immediate formation of a thick precipitate. The reaction was allowed to reach 0 °C, and a suspension of NCS (4.63 g, 32.1 mmol) in THF (20 mL) was added, whereupon the slurry turned purple. After 45 min at rt, the mixture was filtered over SiO_2, eluting with EtOAc. Evaporation, dilution with EtOAc:hexane 1:5 and filtration over SiO_2 gave the 5.0 g (53%) of **31** as a pale brown oil, which was used without further purification. ^{1}H NMR ($CDCl_3$) δ 7.73 (d, J = 4.1 Hz, 1H), 6.92 (d, J = 4.1 Hz, 1H), 5.93–5.75 (m, 2H), 5.25 (q, J = 1.5 Hz, 1H), 5.23–5.13 (m, 3H), 3.82 (s, 2H), 3.16 (d, J = 6.4 Hz, 4H).

5-Aminomethylthiophene-2-sulfonic Acid, *N'*-[4-(3-Chloro-5-trifluoromethyl-pyridin-2-ylamino)butanoyl]-hydrazide (32b). The bisallylsulfonylhydrazide **32a** was synthesized according to the synthesis of **I** using sulfonyl chloride **31**. Isolated yield of **32a**: 4.0 g (98%). ^{1}H NMR (DMSO-d_6) δ 10.0 (d, J = 3.7 Hz, 1H), 9.85 (d, J = 3.7 Hz, 1H), 8.30 (s, 1H), 7.93 (d, J = 2 0.3 Hz, 1H), 7.42 (d, J = 3.7 Hz, 1H), 7.29 (t, J = 5.4 Hz, 1H), 6.94 (d, J = 3.7 Hz, 1H), 5.9–5.67 (m, 2H), 5.26–5.02 (m, 4H), 3.72 (s, 2H), 3.32 (q, J = 7.3 Hz, 2H), 3.03 (d, J = 6.0 Hz, 4H), 2.04 (t, J = 7.7 Hz, 2H), 1.66 (q, J = 7.3 Hz, 2H), MS *m/z* 552.6 (M + H); 550.4 (M − H). A solution of **32a** (4.0 g, 7.25 mmol), *N,N'*-dimethylbarbituric acid (NDMBA 2.8 g, 18.1 mmol), and $Pd(PPh_3)_4$ (148.8 mg, 0.13 mmol) in DCM was degassed with argon. The reaction mixture was stirred for 3 h at rt after which the desired amine **32b** precipitated as its NDMBA salt. The mixture was diluted with EtOAc (200 mL) and hexane (200 mL) and washed with water (3 × 50 mL). The combined aqueous phases were freeze-dried, dissolved in a minimal amount of MeOH, and purified by chromatography (SiO_2, DCM/EtOAc/NH_4OH.aq 80:20:5). The chromatography was repeated twice and gave 2.3 g (67%) of the free amine **32b**, which was dissolved in refluxing EtOAc (80 mL) and cooled to −18 °C to afford 1.7 g (50%) of **32b** as a white powder: ^{1}H NMR (DMSO-d_6) δ 10.02–9.85 (br. s, 1H), 8.24–8.19 (br. s, 1H), 7.85 (d, J = 2.0 Hz, 1H), 7.32 (d, J = 3.8 Hz, 1H), 7.20 (t, J = 5.7 Hz, 1H), 6.82 (d, J = 3.8 Hz, 1H), 5.3–4.3 (br. s, 2H), 3.80 (s, 2H), 3.23 (q, J = 6.7 Hz, 2H), 1.96 (t, J = 7.5 Hz, 2H), 1.57 (quint, J = 7.2 Hz, 2H); MS *m/z* 472 (M + H); Anal. ($C_{15}H_{17}ClF_3N_5O_3S_2$): C, H, N.

4-Chloro-*N*-[(5-{[2-({1-[3-chloro-5-trifluoromethylpyridin-2-yl]piperidin-4-yl}carbonyl)hydrazino]sulfonyl}-2-thienyl)methyl]benzamide (33). **33** was synthesized using **2** as sulfonyl chloride and 3'-chloro-5'-trifluoromethyl-3,4,5,6-tetrahydro-2*H*-[1,2']bipyridinyl-4-carboxylic acid hydrazide according to the synthesis of **I**. Isolated yield after silica gel chromatography (DCM/MeOH 20:1): 97 mg (76%) of a white solid. ^{1}H NMR (DMSO-d_6) δ 10.1 (d, J = 3.7 Hz, 1H), 9.90 (d, J = 3.7 Hz, 1H), 9.35 (t, J = 6.0 Hz, 1H), 8.50 (s, 1H), 8.13 (d, J = 1.9 Hz, 1H), 7.86 (d, J = 8.7 Hz, 2H), 7.47 (d, J = 8.3 Hz, 2H), 7.42 (d, J = 3.7 Hz, 1H), 7.06 (d, J = 3.7 Hz, 1H), 4.61 (d, J = 5.6 Hz, 2H), 3.76 (d, J = 12.8 Hz, 2H), 2.75 (t, J = 11.3 Hz, 2H), 2.39–2.22 (m, 1H), 1.65–1.36 (m, 4H), MS *m/z* 636.2 (M + H); 634.0 (M − H).

General Procedure for Acylation of 32b. Synthesis of 34a–w, 35a–g, 36a–f. A 20 mg/mL solution of **32b** in pyridine/DCM 1:4 was cooled to −40 °C and treated for 1 h with 0.8 equiv of the desired acyl chloride RCOCl. The reaction mixture was brought to room temperature over 30 min. The desired amide was purified by evaporation, dilution in CH_3-CN, and filtration over a SiO_2 pad. The final evaporation afforded the desired amide in typically 50–80% yield and purity > 90%.

General Procedure for Sulfonylation, Carbamoylation of 32b. Synthesis of 37 and 38a–c. A 19 mg/mL solution of **32b** in pyridine/DCM 1:4 was mixed for 10 min with 0.9 equiv of either a sulfonyl chloride RSO_2Cl or an isocyanate RNCO. Evaporation, dilution in CH_3CN, filtration over a SiO_2 pad, and evaporation afforded the desired sulfonamide or urea in ca. 50% yield for the sulfonamides, and ca. 70% for the ureas. Typical purity: 90–95%.

General Procedure for reductive alkylation of 32b. Synthesis of *N'*-({5-[(benzylamino)methyl]-2-thienyl}-sulfonyl)-4-{[3-chloro-5-(trifluoromethyl)pyridin-2-yl]-amino}butanohydrazide (39). A solution of **32b** (20 mg, 0.043 mmol), benzaldehyde (4.5 μL, 0.043 mmol), and AcOH (9.0 μL, 0.16 mmol) in MeOH (1.5 mL) was stirred at rt for 20 min before adding $NaBH(OAc)_3$ (24 mg, 0.113 mmol). After 2 h, dilution with EtOAc (5 mL) and filtration over a silica gel pad afforded 23.4 mg (97%) of the desired amine as a colorless oil. ^{1}H NMR (DMSO-d_6) δ 9.84–9.80 (br. s, 1H), 8.33–8.28 (br. s, 1H), 7.59 (d, J = 2.0 Hz, 1H), 7.46 (d, J = 3.8 Hz, 1H), 7.28–7.15 (m, 5H), 6.83 (d, J = 3.9 Hz, 1H), 5.53 (t, J = 6.0 Hz, 1H), 5.10–4.40 (br. s, 2H), 3.9 (s, 2H), 3.7 (s, 2H), 3.43 (q, J = 6.6 Hz, 2H), 2.17 (t, J = 7.4 Hz, 2H), 1.79 (quint, J = 7.1 Hz, 2H). MS *m/z* 562 (M + H); Anal. ($C_{22}H_{23}ClF_3N_5O_3S_2$): C, H, N.

General Procedure for Solid-Supported Parallel Synthesis of 40a–d, 41a–g, 42a–e, 43a–h, 50l. In a Quest 210 parallel synthesizer 5 mL reaction vessels were placed 2 mL of DCM, 0.5 mL of DMF, 0.165 mmol of amine and polymer-bound morpholine (4 equiv of PS–NMM 2.2 mmol/g). The mixture (in each vessel) was stirred for 30 min at rt after which 1 mL of a stock solution of **2** or **29** in DCM/DMF 1:1 (0.15molar) was added and the mixture was further stirred for 15 h at rt. Polymer-bound aminomethyl polystyrene (2 equiv of PS–NH2 1.3 mmol/g) and polymer-bound isocyanate (2 equiv of PS–NCO 1.5 mmol/g) were added. The mixture was stirred for 5 h at rt and drained through the lower luer manifold. The remaining resins were washed three times with DCM, and the collected solutions were concentrated using a GeneVac parallel concentrator affording **40a–d, 41a–g, 42a–e, 43a–h** typically as solid (yields: 60–98%). Purities ranged from 90 to 99%.

General Procedure for the Synthesis of 44a–k, 45a–e, 46a–d in Case of Two Isomers (as exemplified by the synthesis of *N*-Boc-4-benzotriazolylpiperidines (**44a, 45a**)). To a solution of 1-Boc-4-hydroxypiperidine (5.2 g, 25 mmol), benzotriazole (5.95 g, 50 mmol), and triphenylphosphine (13.7 g, 50 mmol) in 375 mL of THF anhydrous was slowly added DEAD (8.15 mL, 50 mmol) in 250 mL of THF over 3 h. The yellow solution was stirred overnight, THF was evaporated to dryness, and the residue was purified by flash chromatography using cyclohexane/EtOAc 7:3 as eluent. Two major fractions were isolated, of which the first eluting fraction contained 4.5 g (60%) of the 2-isomer *N*-Boc-4-benzotriazol-2-ylpiperidine **45a** ^{1}H NMR ($CDCl_3$) δ 7.87 (d, J = 3.0 Hz, 1H),

7.85 (d, J = 3.0 Hz, 1H), 7.39 (d, J = 3.0 Hz, 1H), 7.37 (d, J = 3.0 Hz, 1H), 4.92 (quint, J = 7.3 Hz, 1H), 4.24 (br d, J = 11.3 Hz, 2H), 3.04 (quint, J = 6.7 Hz, 2H), 1.48 (s, 9H), MS m/z 247 (M-56+H); 203 (M − Boc + H).

Later eluting fractions contained 2.25 g (30%) of the 1-isomer *N*-Boc-4-benzotriazol-1-yl-piperidine **44a**. ^{1}H NMR (DMSO-d_6) δ 8.12 (br s, 1H), 8.01 (d, J = 8.3 Hz, 1H), 7.47 (d, J = 9.0 Hz, 1H), 5.29−5.15 (m, 1H), 3.44 (br d, J = 13.1 Hz, 2H), 3.32 (s, 9H), 3.19 (br t, J = 12.2 Hz, 2H), 2.46−2.22 (m, 4H), MS m/z 203 (M − Boc + H).

General Procedure for the Synthesis of 44a−k, 45a−e, 46a−d in Case of Three Isomers (as exemplified by the synthesis of *N*-Boc-4-chlorobenzotriazolylpiperidines (**44e**, **44f**, **45b**). The synthesis was carried out as described in the protocol for **44a** and **45a**. The crude product was purified by flash chromatography using petroleum ether/EtOAc 7:1. Three major fractions were collected, in which first eluting fractions (R_f=0.5) contained 350 mg (60%) of the 2-isomer *N*-Boc-4-(5-chlorobenzotriazol-2-yl)-piperidine **45b**. ^{1}H NMR (CDCl3) δ 7.84 (d, J = 1.9 Hz, 1H), 7.79 (d, J = 9.0 Hz, 1H), 7.33 (dd, J = 9.0, 1.8 Hz, 1H), 4.89 (quint, J = 7.4 Hz, 1H), 4.23 (br d, J = 11.3 Hz, 2H), 3.04 (quint, J = 6.6 Hz, 2H), 2.34−2.2 (m, 4H), 1.48 (s, 9H), MS m/z 337 (M − Boc + H).

The second eluting fraction (R_f=0.3) contained 114 mg (17%) of *N*-Boc-4-(6-Chlorobenzotriazol-1-yl)-piperidine **44f**. ^{1}H NMR ($CDCl_3$) δ 7.99 (d, J = 8.7 Hz, 1H), 7.56 (d, J = 1.1 Hz, 1H), 7.33 (dd, J = 9.0, 1.88 Hz, 1H), 3.02 (t, J = 12.0 Hz, 2H), 4.84−4.69 (m, 1H), 4.40−4.24 (m, 4H), 2.31 (q, J = 11.9 Hz, 2H), 2.15 (d, J = 2.8 Hz, 2H), 1.50 (s, 9H). Structure was assigned using 2D-NOE experiments. A cross-peak between H^{7arom} and $H^{3piperidine}$ was observed. MS m/z 337 (M − Boc + H).

The third eluting fraction (R_f = 0.2) contained 23 mg (4%) of *N*-Boc-4-(5-chlorobenzotriazol-1-yl)piperidine (**44e**). ^{1}H NMR ($CDCl_3$) δ 8.07 (s, 1H), 7.50 (t, J = 8.8 Hz, 2H), 4.89−4.75 (m, 1), 3.05 (t, J = 12.4 Hz, 2H), 2.33 (q, J = 11.3 Hz, 2H), 2.17 (d, J = 13.1 Hz, 2H), 1.51 (s, 9H), 1.27 (d, J = 6.8 Hz, 2H). Structure was assigned using 2D-NOE experiments. A cross-peak between H^{7arom} and $H^{3piperidine}$ was observed. MS m/z 337 (M − Boc + H).

General Procedure for the Synthesis of 47a−k, 48a−e, 49a−d (as exemplified by the synthesis of 4-benzotriazolylpiperidinium trifluoroacetates (**47a**, **48a**)). A solution of **44a** (2.25 g, 7.45 mmol) in 125 mL of DCM was treated with 25 mL of TFA. The reaction was stirred for 90 min at rt and evaporated to dryness. The oily residue was treated with diethyl ether several times, upon which 2.23 g of the corresponding trifluoroacetate of **47a** precipitated (99%) as a colorless solid, which was further neutralized with NH_4OH. ^{1}H NMR (DMSO-d_6) δ 8.02 (d, J = 8.4 Hz, 1H), 7.92 (d, J = 8.4 Hz, 1H), 7.52 (t, J = 8.4 Hz, 1H), 7.38 (t, J = 8.4 Hz, 1H), 4.91 (m, 1H), 3.09 (d, J = 12.4 Hz, 2H), 2.69 (t, J = 12.5 Hz, 2H), 2.19−1.97 (br. m, 5H). MS m/z 203 (M + H), 201.2 (M − H).

Accordingly 4-benzotriazol-2-ylpiperidinium trifluoroacetate (**48a**) could be accessed in quantitative yield. ^{1}H NMR ($CDCl_3$) δ 8.95 (br s, 1H), 8.73 (br s, 1H), 7.94 (d, J = 3.4 Hz, 1H), 7.92 (d, J = 3.0 Hz, 1H), 7.45 (d, J = 3.4 Hz, 1H), 7.43 (d, J = 3.0 Hz, 1H), 5.28−5.14 (m, 1H), 3.45 (d, J = 12.8 Hz, 2H), 3.21 (t, J = 10.1 Hz, 2H), 2.48−2.28 (m, 4H), MS m/z 203 (M + H), 201.2 (M − H).

Syntheses of 4-Benzotriazolylpiperidinesulfonamides 50a−k, 51a−e, 52a−d (as exemplified by the synthesis of *N*-[5-(4-benzotriazol-1-ylpiperidine-1-sulfonyl)thiophen-2-ylmethyl]-4-chlorobenzamide (**50a**) and *N*-[5-(4-benzotriazol-2-ylpiperidine-1-sulfonyl)thiophen-2-ylmethyl]-4-chlorobenzamide (**51a**)). To a solution of **44a** (2.23 g, 7 mmol) and DIEA (3.6 mL, 21 mmol) in 45 mL of DCM was added during 1 h a solution of **2** (2.2 g, 6.3 mmol) in DCM/DMF 9:1 (100 mL). The reaction mixture was stirred for 3 h at rt. The organic layer was washed with 0.1 N HCl and extensively washed with brine. After drying over $MgSO_4$, the solvent was evaporated to yield 3.3 g of crude **50a**. The crude product was recrystallized from DCM/cyclohexane to yield 2.52 g (70%) of pure **50a**. ^{1}H NMR (DMSO-d_6) δ 9.40 (t, J = 5.6 Hz, 1H), 8.02 (d, J = 8.3 Hz, 1H), 7.91 (d, J = 8.3 Hz, 2H), 7.87 (d, J = 8.3 Hz, 1H), 7.61−7.55 (m, 3H), 7.52 (t, J = 8.3 Hz, 1H), 7.38 (t, J = 7.9 Hz, 1H), 7.23 (d, J = 3.7 Hz, 1H), 5.00 (quint, J = 7.34 Hz, 1H), 4.70 (d, J = 5.6 Hz, 2H), 3.78 (d, J = 12.0 Hz, 2H), 2.79−2.64 (m, 2H), 2.3−2.16 (m, 4H), MS m/z 516.0 (M + H); 514.0 (M − H). Anal. ($C_{23}H_{22}ClN_5O_3S_2$) C, H, N.

Similarly, *N*-[5-(4-benzotriazol-2-ylpiperidine-1-sulfonyl)-thiophen-2-ylmethyl]-4-chlorobenzamide (**51a**) was prepared in 42% yield. ^{1}H NMR (DMSO) δ 9.38 (t, J = 5.6 Hz, 1H), 7.94−7.83 (m, 4H), 7.56 (d, J = 8.6 Hz, 2H), 7.53 (d, J = 3.7 Hz, 1H), 7.5−7.37 (m, 2H), 7.20 (d, J = 3.7 Hz, 1H), 5.04−4.9 (m, 1H), 4.68 (d, J = 5.6 Hz, 2H), 3.68 (br d, J = 12.4 Hz, 2H), 2.76 (br t, J = 12.6 Hz, 3H), 2.39 (br d, J = 13.1 Hz, 2H), 2.22 (br t, J = 11.1 Hz, 2H), MS m/z 516.0 (M + H); 514.0 (M − H); Anal. ($C_{23}H_{22}ClN_5O_3S_2$) C, H, N.

4-Chloro-*N*-{5-[4-(2-trifluoromethylbenzoimidazol-1-yl)piperidine-1-sulfonyl]thiophen-2-ylmethyl}benzamide (50m). *N*-Boc-4-aminopiperidine (500 mg, 2.5 mmol) and 1-fluoro-2-nitrobenzene (210 μL, 2 mmol) where heated in the presence of DIEA (1 mL, 6.25 mmol) in DMF at 70 °C for 15 h. After aqueous workup a crude yellow solid was purified on silica gel using petroleum ether/EtOAc 5:1 as eluent to yield 500 mg (78%) of *N*-Boc-4-(2-nitrophenylamino)-piperidine. MS m/z 222.2 (M − Boc + H). 250 mg (0.78 mmol) of the secondary nitroaniline derivative was exposed to hydrogen-gas flow in EtOH in the presence of palladium on charcoal. After 1 h the yellow color completely disappeared. The solution was filtered through a microfilter (0.45 μm) yielding a reddish solution which after solvent evaporation gave 170 mg (75%) of *N*-Boc-4-(2-aminophenylamino)-piperidine. MS m/z 194.2 (M − Boc + H). The 2-aminoaniline derivative (120 mg, 0.41 mmol) was dissolved in 10 mL of DCM, to which 2 mL of TFA was slowly added. The solution was stirred for 3 h at rt until the deprotection/cyclization of the benzimidazole was completed as monitored by LC-MS. The reaction was evaporated to dryness, and the oily residue was treated with diethyl ether affording 70 mg (45%) of a pink precipitate which was identified as 1-piperidin-4-yl-2-trifluoromethyl-1*H*-benzimidazole trifluoroacetate. ^{1}H NMR (DMSO-d_6) δ 8.70 (br s, 1H), 8.51 (br s, 1H), 8.05 (d, J = 8.3 Hz, 1H), 7.87 (d, J = 8.3 Hz, 1H), 7.50 (t, J = 7.3 Hz, 1H), 7.40 (t, J = 7.5 Hz, 1H), 4.94−4.77 (m, 1H), 3.59−3.13 (m, 7H), 2.78−2.59 (m, 2H), 2.09 (br d, J = 11.6 Hz, 2H), MS m/z 269.8 (M − Boc + H).

The trifluoroacetate was coupled to the sulfonyl chloride **2** as described in the protocol for the syntheses of 4-benzotriazolylpiperidinesulfonamides **50a−k**, **51a−e**, **52a−d**. **50m** (13 mg, 60%) was isolated after flash chromatography using cyclohexane/EtOAc as eluent. ^{1}H NMR (DMSO-d_6) δ 9.40 (t, J = 6.0 Hz, 1H), 7.90 (d, J = 8.6 Hz, 2H), 7.82 (d, J = 7.5 Hz, 1H), 7.68 (d, J = 8.3 Hz, 1H), 7.61−7.53 (m, 3H), 7.47−7.31 (m, 2H), 7.25 (d, J = 3.7 Hz, 1H), 4.71 (d, J = 5.6 Hz, 2H), 4.67−4.49 (m, 1H), 3.85 (br d, J = 12.2 Hz, 2H), 2.80 (br t, J = 11.4 Hz, 2H), 2.57−2.37 (m, 2H), 1.99 (d, J = 10.3 Hz, 2H), MS m/z 583.1 (M + H); 581.5 (M − H).

Biological Methods. 1. rJNK3 and rJNK2 Enzymatic Assay. JNK3 and/or -2 assays were performed in 96-well microtiter (MT) plates, by incubation of 0.5 μg of recombinant, preactivated GST-JNK3 or GST-JNK2 with 1 μg of recombinant, biotinylated GST-c-Jun and 2 μM $^{33}\gamma$-ATP (2 nCi/μL), in the presence or absence of sulfonamide inhibitors in a reaction volume of 50 μL containing 50 mM Tris-HCl, pH 8.0; 10 mM $MgCl_2$; 1 mM dithiothreitol, and 100 μM $NaVO_4$. The incubation was performed for 120 min at rt and stopped upon addition of 200 μL of a solution containing 250 μg of streptavidin-coated SPA beads (Amersham, Inc.), 5 mM EDTA, 0.1% Triton X-100, and 50 μM ATP, in phosphate saline buffer. After incubation for 60 min at rt, beads were sedimented by centrifugation at 1500g for 5 min, resuspended in 200 μL of PBS containing 5 mM EDTA, 0.1% Triton X-100, and 50 μM ATP, and the radioactivity was measured in a scintillation β counter, following sedimentation of the beads as described above. By substituting GST-c Jun for biotinylated GST-$_1$ATF$_2$ or myelin basic protein, this assay could also be used to

measure inhibition of preactivated p38 and ERK MAP kinases, respectively.

2. Sympathetic Neuron Culture (SCG) and Survival Assay. Sympathetic neurons from superior cervical ganglia (SCG) of new-born rats (p0, p3) were dissociated in 2% Dispase, plated at a density of 4×10^4 cells/mL in 48-well MT plates coated with rat tail collagen and cultured in Leibowitz medium containing 5% rat serum, 0.75 μg/mL NGF 7S (Boehringer Mannheim Corp., Indianapolis, IN) and arabinosine C 10^{-5} M. Cell death was induced at day 4 after plating by exposing the culture to medium containing 10 μg/mL of anti NGF antibody (Boehringer Mannheim Corp., Indianapolis, IN) and no NGF or arabinosine C, in the presence or absence of sulfonamide inhibitors in 1% DMSO. Twenty four hours after cell death induction medium was removed again, and cells were fed with initial medium (without arabinosineC). Twenty four hours later determination of cell viability was performed by incubation of the culture for 30 min, at 37 °C in 0.5 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). After incubation in MTT, cell medium was removed, cells were resuspended in DMSO and transferred to a 96-well MT plate, and cell viability was evaluated by measuring optical density at 590 nm.

Acknowledgment. We thank the Analytical Chemistry group for the support in physicochemical parameters determination and Dr. Jasna Klicic for reading and correcting to manuscript.

References

(1) Cobb, M. H.; Goldsmith, E. J. How MAP kinases are regulated. *J. Biol. Chem.* **1995**, *270*, 14843–14846.
(2) Minden, A.; Karin, M. Regulation and function of the JNK subgroup of MAP kinases. *Biochim. Biophys. Acta* **1997**, *1333*, F85–104.
(3) Barr, R. K.; Bogoyevitch, M. A. The c-Jun N-terminal protein kinase family of mitogen-activated protein kinases (JNK MAPKs). *Int. J. Biochem. Cell Biol.* **2001**, *33*, 1047–1063.
(4) Gupta, S.; Barrett, T.; Whitmarsh, A. J.; Cavanagh, J.; Sluss, H. K.; Derijard, B.; Davis, R. J. Selective interaction of JNK protein kinase isoforms with transcription factors. *EMBO J.* **1996**, *15*, 2760–2770.
(5) Hibi, M.; Lin, A.; Smeal, T.; Minden, A.; Karin, M. Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. *Genes Dev.* **1993**, *7*, 2135–2148.
(6) Whitmarsh, A. J.; Shore, P.; Sharrocks, A. D.; Davis, R. J. Integration of MAP kinase signal transduction pathways at the serum response element. *Science* **1995**, *269*, 403–407.
(7) Chow, C. W.; Rincon, M.; Cavanagh, J.; Dickens, M.; Davis, R. J. Nuclear accumulation of NFAT4 opposed by the JNK signal transduction pathway. *Science* **1997**, *278*, 1638–1641.
(8) Milne, D. M.; Campbell, L. E.; Campbell, D. G.; Meek, D. W. p53 is phosphorylated in vitro and in vivo by an ultraviolet radiation-induced protein kinase characteristic of the c-Jun kinase, JNK1. *J. Biol. Chem.* **1995**, *270*, 5511–5518.
(9) Mohit, A. A.; Martin, J. H.; Miller, C. A. p493F12 kinase: a novel MAP kinase expressed in a subset of neurons in the human nervous system. *Neuron* **1995**, *14*, 67–78.
(10) Dong, C.; Yang, D. D.; Wysk, M.; Whitmarsh, A. J.; Davis, R. J.; Flavell, R. A. Defective T cell differentiation in the absence of Jnk1. *Science* **1998**, *282*, 2092–2095.
(11) Yang, D. D.; Conze, D.; Whitmarsh, A. J.; Barrett, T.; Davis, R. J.; Rincon, M.; Flavell, R. A. Differentiation of CD4+ T cells to Th1 cells requires MAP kinase JNK2. *Immunity* **1998**, *9*, 575–585.
(12) Sabapathy, K.; Hu, Y.; Kallunki, T.; Schreiber, M.; David, J. P.; Jochum, W.; Wagner, E. F.; Karin, M. JNK2 is required for efficient T-cell activation and apoptosis but not for normal lymphocyte development. *Curr. Biol.* **1999**, *9*, 116–125.
(13) Tournier, C.; Hess, P.; Yang, D. D.; Xu, J.; Turner, T. K.; Nimnual, A.; Bar-Sagi, D.; Jones, S. N.; Flavell, R. A.; Davis, R. J. Requirement of JNK for stress-induced activation of the cytochrome c-mediated death pathway. *Science* **2000**, *288*, 870–874.
(14) Yang, D. D.; Kuan, C. Y.; Whitmarsh, A. J.; Rincon, M.; Zheng, T. S.; Davis, R. J.; Rakic, P.; Flavell, R. A. Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the Jnk3 gene. *Nature* **1997**, *389*, 865–870.
(15) Lin, A. Activation of the JNK signaling pathway: Breaking the brake on apoptosis. *Bioessays* **2003**, *25*, 17–24.
(16) Manning, A. M.; Davis, R. J. Targeting JNK for therapeutic benefit: from junk to gold? *Nat. Rev. Drug Discovery* **2003**, *2*, 554–565.
(17) Bennett, B. L.; Sasaki, D. T.; Murray, B. W.; O'Leary, E. C.; Sakata, S. T.; Xu, W.; Leisten, J. C.; Motiwala, A.; Pierce, S.; Satoh, Y.; Bhagwat, S. S.; Manning, A. M.; Anderson, D. W. SP600125, an anthrapyrazolone inhibitor of Jun N-terminal kinase. *Proc. Natl. Acad. Sci. U.S.A* **2001**, *98*, 13681–13686.
(18) Kennedy, N. J.; Davis, R. J. Role of JNK in tumor development. *Cell Cycle* **2003**, *2*, 199–201.
(19) Bain, J.; McLauchlan, H.; Elliott, M.; Cohen, P. The specificities of protein kinase inhibitors: an update. *Biochem. J.* **2003**, *371*, 199–204.
(20) Scapin, G.; Patel, S. B.; Lisnock, J.; Becker, J. W.; LoGrasso, P. V. The structure of JNK3 in complex with small molecule inhibitors: structural basis for potency and selectivity. *Chem. Biol.* **2003**, *10*, 705–712.
(21) Dumas, J. Protein kinase inhibitors: emerging pharmacophores 1997–2000. *Expert Opin. Ther. Pat.* **2001**, *11*, 405–429.
(22) Cremlyn, R.; Ellis, L.; Pinney, A. Chlorosulfonation of N-benzyl carboxamides. *Phosphorus, Sulfur Silicon Relat. Elem.* **1989**, *44*, 167–175.
(23) Wityak, J.; Fevig, J. M.; Jackson, S. A.; Johnson, A. L.; Mousa, S. A.; Parthasarathy, A.; Wells, G. J.; DeGrado, W. F.; Wexler, R. R. Synthesis and antiplatelet activity of DMP 757 analogues. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2097–2100.
(24) Huang, J.; Widlanski, T. S. Facile synthesis of sulfonyl chlorides. *Tetrahedron Lett.* **1992**, *33*, 2657–2660.
(25) Reynolds, R. C.; Crooks, P. A.; Maddry, J. A.; Akhtar, M. S.; Montgomery, J. A.; Secrist, J. A., III Synthesis of thymidine dimers containing internucleoside sulfonate and sulfonamide linkages. *J. Org. Chem.* **1992**, *57*, 2983–2985.
(26) Raju, B.; Wu, C.; Kois, A.; Verner, E.; Okun, I.; Stavros, F.; Chan, M. F. Thiophenesulfonamides as endothelin receptor antagonists. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2651–2656.
(27) Garro-Helion, F.; Merzouk, A.; Guibe, F. Mild and selective palladium(0)-catalyzed deallylation of allylic amines. Allylamine and diallylamine as very convenient ammonia equivalents for the synthesis of primary amines. *J. Org. Chem.* **1993**, *58*, 6109–6113.
(28) Lipinski, C. A. Drug-like properties and the causes of poor solubility and poor permeability. *J. Pharmacol. Toxicol. Methods* **2001**, *44*, 235–249.
(29) Katritzky, A. R.; Oniciu, D. C.; Ghiviriga, I. The Mitsunobu reaction: an alternative synthesis of 1-(primary alkyl)benzotriazoles. *Synth. Commun.* **1997**, *27*, 1613–1621.
(30) Legos, J. J.; McLaughlin, B.; Skaper, S. D.; Strijbos, P. J.; Parsons, A. A.; Aizenman, E.; Herin, G. A.; Barone, F. C.; Erhardt, J. A. The selective p38 inhibitor SB-239063 protects primary neurons from mild to moderate excitotoxic injury. *Eur. J. Pharmacol.* **2002**, *447*, 37–42.

JM031112E